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Will the JET age ever come?

THERE is only one thing more frustrating than the well-known impossibility of learning precisely when particular European Community decisions are made. That is learning that they are not made. Last week's debacle at the Energy and Research Council meetings in Brussels was nothing if not frustrating.

Two major issues, among many others, confronted the Energy Ministers: the minimum safeguard price (msp) for oil, to protect investment in the North Sea and in alternative energies; and implementation of an agreed Euratom loans scheme for construction of nuclear power stations. The two have always been linked, with Britain refusing to sanction implementation of the loans scheme without agreement on the msp, and France preferring selective support to the msp.

Only one issue, or so it seemed, confronted the Research Ministers: where to site JET, the Community's fusion project. On that decision also hung the implementation of the four-year programme of the EEC's Joint Research Centre (JRC), agreed in principle months before. Since the JRC establishment at Ispra stood to benefit by the programme, its candidacy for JET had weakened next to Culham in Britain and Garching in West Germany, but France had pushed intensively for Cadarache.

With one European Commissioner (Guido Brunner) now handling both the Energy and Research portfolios, and growing talk of JET as an energy rather than as a research project, the idea of agreement in one council permitting agreement in the other emerged as soon as the two council meetings were set for the same day. Sure enough, progress in the Energy Council produced British concessions on the

msp, and alternative schemes will now be examined. Implementation of the Euratom loans scheme was then agreed, and the hope was accordingly expressed that this would assist the Research Council.

It didn't. The Research Council could not agree on a site for JET, for the new but genuine enough reason expressed by France that critical organisational and financial details concerning the way the project would be run were not finalised. What is astonishing is that these matters had not been agreed in the interminable meetings held at all levels over the previous 18 months. What is surprising is that the Council nodded through the four-year JRC programme anyway.

Apart from the embarrassment (especially noticeable in Britain) that goes with such developments, the result is that a decision on JET is no longer contingent upon a tangled web of linked issues. That may mean an early decision if the new difficulties can be clarified, but no one is counting on it. Crazy, the only action has again been inaction.

Though this is an era of international competition and energy crisis, our worry is not whether or when a breakthrough on fusion comes, and certainly not who achieves it. Science does transcend frontiers. Our worry is the uncertainty which indecision generates. JET is an experiment, not a reactor, and its research team deserves better. Now may be an appropriate time to examine the thinking which makes international scientific projects matters of national prestige. Otherwise the Community's processes, though favouring compromise and calculated not to alienate, run the risk of doing the opposite by too faithfully serving governments rather than people. □

Science as a profession

THE founding fathers of the Royal Institute of Chemistry, which was last week celebrating its centenary, envisaged a body which would act for chemists rather than the Royal Colleges do for medical men or as the Inns of Court do for lawyers. In spite of a distinguished record in many respects, the Institute has in that aim been totally unsuccessful. Neither chemists, nor indeed any group of professional scientists, form a comparable profession. Scientists and engineers are neither as secure nor as confident nor as well remunerated as doctors or lawyers. Society may view this favourably; scientists find it uncomfortable.

The difference must in part be because most scientists are neither self-employed nor fee-earning, and may consequently find themselves out of work in a recession. Sir Harold Wilson, in praising chemists at the centenary celebrations, cited the case of his own father, a dye chemist, who became Winston Churchill's election agent while out of work for two years—perhaps persuading the infant Wilson of the surer future available in Parliament. Yet, although not as secure as members of the other liberal professions, professional scientists are expected to have similar standards of ethics and behaviour. In addition to integrity in research and loyalty to an employer, particularly as regards confidentiality, scientists are expected to speak out if their work throws up any environmental or health hazards, even though such revelations may damage

their employing company.

Some of the disquiet scientists feel towards government was expressed last week by the President of the Royal Institute of Chemistry when, in reply to the former Prime Minister, he voiced the irritation at the currently fashionable expression "the two sides of industry". The point is, research scientists in industrial laboratories do not see themselves as being on either side. They feel their views are not being sought, much less heard or heeded, on major issues. When British opinion is being moulded to support wealth creation, they believe that as major wealth creators they are not beneficiaries of that support.

Crucially, scientists see the innovations of research and development as the source of profit of leading companies—failures to exploit science (as with the loss of patents on antibiotics or jet engines) not being the fault of the originators. Chemists in particular are disgruntled because they feel largely responsible for the country's thriving chemical industry and its near relatives in petrochemicals, pharmaceuticals and so on. The companies concerned do not include any of the famous lame ducks. They are almost never involved in industrial disputes. They are highly scientifically innovative. They make the profits which are taxed to support other industries unable to stand up to international competition because, in many cases, of their small research investment.



In spite of this scientific and commercial success, the people who carry the burden in research do not see themselves in a secure profession. Both output of and demand for chemists has declined during this decade and there is evidence that the quality of the newly qualified has dropped. The medical and legal professions, on the other hand, control both entry and the right to practice as well as the discipline of their professions, thus evading the cyclical imbalance in supply and demand so often felt by scientists.

Science as a profession is in a weak position. The best scientists are evenly spread over the country and not concentrated in the capital with day to day access to Whitehall.

Salary structures are strongly influenced by central government because the latter is the major employer, basing its rewards on comparability with industry which in turn compares with civil service salaries. It is a small but vicious circle which depresses salaries.

If the recent Bullock proposals for worker-directors in Britain are implemented along anything like the lines suggested in the majority report, the new directors are unlikely to include research scientists. Yet scientists are a major wealth-creating source. They may not continue to provide for the community if their views are not sought or are disregarded, and certainly if they are driven overseas by the tax structure. □

A parable on recombination

DeWitt Stetten offers some personal thoughts on the management of hazards in recombinant DNA research

SCIENTISTS and nonscientists are currently polarised in their assessments of the hazards attached to the creation and handling of recombinant DNA molecules. Whereas the hazards are of unknown dimensions, the anxieties are clearly great: but the magnitude of the anxiety is a very imperfect measure of the magnitude of the hazard. Among the diverse views we are seeking an area of possible agreement or, better yet, an area of truth.

Truth is sometimes found in unexpected places. It may be recalled that Jonathan Swift, famed satirist and author of *Gulliver's Travels*, while serving as Dean of St Patrick's in Dublin, considered the disastrous problem of the famine among the Irish peasants which was aggravated by the high birth rate and the abundance of children. He analysed the problem in 1729 in an essay entitled 'Modest Proposal for Preventing the Children of Poor People from being a Burden to their Parents or the Country,' in which, with all pretense of reasonableness, he concluded that the entire problem could be solved if the people of Ireland ate the children. In view of the evident success of Swift's satire, I am taking the liberty of presenting here a parable which I call 'Genetic Recombination—A Modest Proposal'.

After all, recombinant DNA technology is a special case of a far more general phenomenon—that of genetic recombination itself, where genetic material from two sources is repeatedly combined within a single cell. It happens when an oncogenic virus invades a mammalian cell, or when a bacteriophage invades a bacterium. It happens when two bacteria enter into conjugation and transfer plasmid DNA from one to another. In the laboratory it can be achieved through the hybridisation of eukaryote cells from widely differing species, and it is of course the very basis of sexual reproduction.

A form of this kind of experimentation which has been popular for a very long time indeed results in the fertilisation of the human ovum. I refer to this exercise as an experiment, because it always is experimental in that the outcome is not known *a priori*. At this time we can not even foretell the sex of the offspring, much less any of the details of its anatomy or character. Yet the experiment, whereas it provides great possibilities of useful and benevolent results, does have a real hazard. Text-books of obstetrics contain many engrossing photographs of anatomical monsters which have resulted from this process, but it seems to me that the very worst monsters, the behavioural ones, are not included. These may be divided into two classes—the societal monsters such as gangsters, typified by Al Capone, and the international monsters, exemplified by Adolf Hitler.

Clearly, some of these monsters possess a survival advantage over the rest of us. One has merely to note the

direction in which the machine gun which the gangster carries is pointed to ascertain which party is in the better position to survive the encounter. Of particular interest is the experiment conducted nearly ninety years ago by the parents of Adolf Hitler. The maternal ovum may be construed in this case as the host, while the paternal sperm clearly was the vector, bearing as it did a charge of DNA which was foreign to the host. The conceptus which resulted was carefully cloned under reasonably sterile and controlled conditions, and there arose an individual who became, directly or indirectly, responsible for the premature deaths of 4×10^7 human beings in the course of about eight years. This mortality is certainly large, even in the parlance of the epidemiologist.

When scientists first began to appreciate the danger attached to the fertilisation of human ova, they directed attention to the matter and called for an immediate moratorium. Since the hazard seemed to be of enormous proportions, the federal government soon became involved; and by and by, a Presidential commission was established to consider the problem and to determine its best resolution. With the moratorium still in effect, one alternative solution proposed was to sterilise all adults, male and female. It was forecast that this procedure would probably work, but it would be quite costly. For this reason, the General Accounting Office, which has the responsibility of surveying costly federal projects, gave its attention to the matter and pointed out that sterilising of either the male or the female population would probably achieve the same desired effect at half the cost. To decide whether to sterilise all males or all females, a special panel of the Equal Employment Opportunity Commission was convened which, with Solomonic wisdom, recommended that since there were approximately equal numbers of males and females in the population at risk, it would appear appropriate to sterilise half of the males and half of the females.

A small experiment was conducted on normal volunteers to test the effectiveness of this procedure, and it was found, for reasons which are still unclear, that this procedure would not yield the desired result. After considerable deliberation, the commission recommended that experiments in human impregnation might proceed with caution in certain designated centres, provided steps were taken to preclude pollution of the environment. It was determined that experiments could be conducted under P4 physical containment with all the manipulations to be carried out in class III safety cabinets which, in the usual fashion, would have their entry and exit ports protected either by autoclaves or by incinerators. Only in this way could the public be assured that no monsters would escape to pollute the environment and damage the ecology. The econiche which we so comfortably occupy would not be threatened by another generation which predictably would perform less nobly than we had done. □

NASA's Space Shuttle

'Re-usability' is the catchword

Having generated plenty of controversy, the Shuttle is now being proven. **Colin Norman** reports

IF you have a piece of hardware weighing up to 65,000 pounds that you would like to put into Earth orbit after 1980, the National Aeronautics and Space Administration (NASA) would be only too pleased to rent you the Space Shuttle for \$20 million. Or if your requirements are more modest—a 200-pound experimental package which doesn't need special handling, for example—NASA would be glad to take it aloft for as little as \$3,000. Although some advance notice and a small deposit would be required, it shouldn't be too difficult to make a booking because there is likely to be considerable spare room in the Shuttle when it eventually flies.

With three years to go before the Shuttle is scheduled to begin full service, NASA is starting to drum up business. A list of fees to be charged to such users as the Department of Defense, foreign governments, commercial operations, and individual researchers has recently been drawn up, and NASA officials have developed an ambitious flight plan calling for as many as 60 shuttle launches a year in the late 1980s.

The flight plan indicates that NASA has not changed its basic thinking on the Shuttle since it won approval for the programme after a bitter fight in the early 1970s. NASA officials are still confident, in short, that the Shuttle will revolutionise space activities by sharply reducing the cost of launching payloads and providing routine access to space. If the flight plan is remotely accurate, in fact, it would represent a staggering increase in the space programme, resulting in more activity than during the height of the Apollo era. Many of those who fought the Shuttle programme in its early stages, including some prominent space scientists, remain sceptical of NASA's projections, however.

The design of the Shuttle was determined in 1972, following an intense struggle between NASA and the Office of Management and Budget. NASA had originally wanted to build a fully re-usable system, consisting of an orbiter and a booster rocket, both of which would be piloted back to Earth and re-used, but in the end it had to settle for an economy model which will be only partially re-usable. It will consist of an Orbiter mounted atop an external fuel tank and strapped to two booster rockets. Soon after launch, the

boosters will separate and be parachuted into the ocean for recovery, refurbishing and re-use. The external fuel tank will be discarded in the upper atmosphere where it will burn up, and the Orbiter, after completing its mission, will re-enter the atmosphere and glide to a landing like an aircraft.

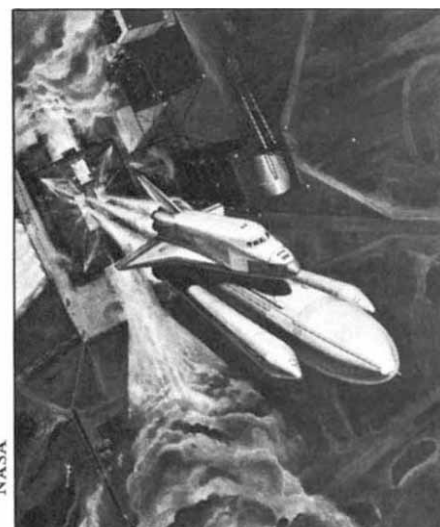
Launches and landings will take place at two locations, Cape Canaveral in Florida and Vandenberg air force base in California. When launched due east from Canaveral, the Shuttle will be capable of delivering 65,000 pounds into a 185 km orbit, or 25,000 pounds into a 500 km orbit at 55 degrees. Missions requiring polar orbits will be launched from Vandenberg because such launches from Canaveral would involve flying over heavily populated areas soon after launch. The Shuttle can deliver 32,000 pounds into a 185 km polar orbit.

The Shuttle itself is capable of reaching only low Earth orbits, and thus an extra piece of gadgetry is needed to hoist communications satellites into higher orbits and to fire planetary spacecraft out of the Earth's gravitational field. Again, NASA was originally planning to develop a re-usable rocket, called a 'Space Tug', for those tasks, but it has had to settle for a cheaper, disposable system, at least for the time being. It consists of a solid-fuelled rocket, called the Interim Upper Stage (IUS), which will be launched from a Shuttle in orbit; the IUS is being developed by the Air Force. Though NASA officials claim that they still hope to build the Space Tug someday, there is some doubt about whether that day will ever come.

Costs go up

When the Office of Management and Budget finally approved NASA's plan in 1972, the estimated cost of developing the Shuttle was about \$5,200 million. The latest projections put the total research and development costs at about \$6,800 million, of which about \$4,500 million will have been spent by the end of this year. Virtually all the increase is accounted for by inflation. In addition, NASA reckons that it will cost about \$2,500 million to produce five orbiters and associated boosters and fuel tanks, bringing the total research, development and production costs to some \$9,300 million.

So far, development of the Shuttle is proceeding on schedule and no major



Shuttle: take-off to recovery

snags have been encountered, which is an impressive record for such a mammoth undertaking. The first Orbiter was unveiled last September and, thanks to a massive mail campaign from *Star Trek* fans, it was named the Enterprise. It is now being flight tested in the atmosphere, riding piggyback on a Boeing 747, and in July or August it will be cast loose at 22,000 feet to glide on its own to a landing at Canaveral. The first orbital test flight is scheduled for March 1979, and if all goes according to plan, it will be ready for full operation in the spring of 1980. Development of the IUS is also proceeding apace, and it is also supposed to be ready by 1980.

After the Office of Management and Budget gave its approval to the Shuttle in 1972, the real battle began. NASA's cost estimates and projected savings for the system were subjected to severe criticism by a group of Senators led by Walter Mondale, now Vice President, who took their cues from a number of prominent space scientists and systems analysts. They included Thomas Gold, Professor of Astrophysics at Cornell, James van Allen, Professor of Physics at the University of Iowa, George Rathjens, Professor of Political Science at MIT, and Brian O'Leary, a former astronaut.

The nub of the debate was this. NASA claimed that the Shuttle would more than pay for itself over its projected 12-year lifetime because it would be able to deliver payloads into orbit much cheaper than expendable rockets could. Critics of the system argued, however, that NASA was assuming an unrealistically high launch rate with the Shuttle, and that the cost comparisons didn't properly take into account the research and development costs of the system. Another objection was that the costs of developing and building the Shuttle would soak up so

much of NASA's budget that many space science and applications projects would get squeezed out. The debate raged for about a year, but died down when Congress finally sided with NASA and voted the money to begin developing the system.

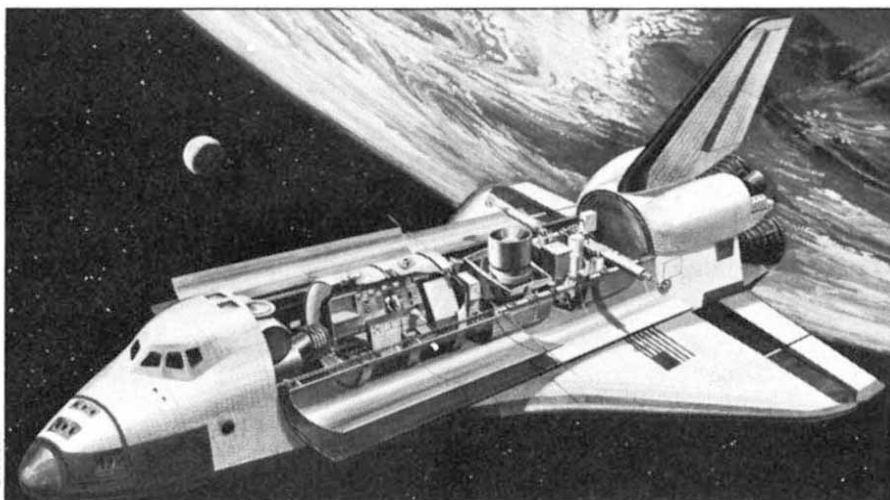
At least one of the critics' complaints has already proven correct. While NASA's budget has remained approximately constant in real terms since 1971, spending on the Shuttle has climbed from less than \$100 million to more than \$1,300 million a year. As a result, there have been very few new space science projects started: some have been scrapped (such as the grand tour of the outer planets), others (such as the High Energy Astronomy Observatory) have been pared, and still others (such as the Large Space Telescope) have been deferred. As O'Leary noted last week, "Our dire scenario has been played out".

Nevertheless, since the project got under way in earnest, NASA officials have been working out a flight plan for the Shuttle based on an assumption that NASA's budget will stay roughly constant in terms of purchasing power over the next 10-15 years, and they have come up with a flight plan very similar to the one which was used to justify the Shuttle in the first place. It should be noted that the plan doesn't represent firm commitments to develop or build a set number of satellites; it is simply a planning document.

NASA calculates that there will be about 560 separate Shuttle flights between 1980 and 1991, with an average of about 60 flights a year between 1985 and 1991. With the massive payload capability of the Shuttle, that represents a very large increase in space activities. According to the flight plan, the breakdown between users will be NASA, 267; other US government agencies, 28; US commercial operations, 60; foreign organisations, 65; Department of Defense, 109. When broken down by type of mission, the plan calls for 229 Spacelab flights, 35 launches of large structures, 168 launches involving upper stages, and 97 launches of free-flying satellites.

Defense's satellites

Aside from the very considerable size of the programme represented by the flight plan, the number of launches envisaged by the Department of Defense is interesting. Though the nature of the defence missions is classified, some two-thirds of them would require use of upper stages, indicating that they are communications satellites or other satellites in geosynchronous orbits. It has also been speculated that the Shuttle's ability to manoeuvre in space will give it the capability of studying, or even capturing, enemy



The Shuttle's Spacelab configuration



Shuttle takes a trip, March 1977

satellites in low orbits.

As for the fees to be charged to users, NASA has recently circulated for comments a proposal which would allow NASA to recoup the operating costs for the Shuttle. The fees would not, however, include payment for a share of the research, development and production costs. Briefly, NASA has proposed that a fee of \$20 million would be charged for chartering the entire Shuttle for a single launch. The Department of Defense and foreign governments which have contributed to the Shuttle development would get a discount, as would US commercial operations. Users who require only a part of the Shuttle capacity would pay on a pro-rated basis with the smallest experimental packages which require no manual attention getting in for only \$3,000 apiece.

NASA reckons that those fees represent about a 40% discount in launch costs when compared with the use of expandable rockets. Asked whether commercial organisations are showing any interest in booking space on the Shuttle, one NASA official said that he has noticed a recent awakening of interest on the part of payload manufacturers. "At one time they were between lukewarm and disinterested in

the Shuttle", he said, "but I have seen a definite reversal in attitude".

As for space scientists, the Shuttle seems to have generated some resentment because of the fact that its development squeezed out a number of scientific missions. Nevertheless, several former critics of the Shuttle contacted last week all agreed that, with nearly \$500 million already spent it would be folly to seek to cancel the programme at this stage. And the Space Science Board of the National Academy of Sciences said in a report published in 1974 that the Shuttle "can be an important asset to scientific research in and beyond the 1980s".

At least one former critic of the Shuttle has also recently been converted into a supporter. Brian O'Leary said last week that although he still believes that NASA's justification of the project is seriously flawed, the Shuttle "by good luck" would be a useful transport vehicle for the possible establishment of space manufacturing systems using lunar materials, to construct space habitats and solar power stations. O'Leary, who is now working with Dr Gerard O'Neill at Princeton exploring the concept of space habitats, said that he now supports the Shuttle for that reason. □

ESA's Spacelab

Research is the watchword

Spacelab offers scientists unique conditions for future research. **Stuart Sharrock** describes how

THE pioneer phase of manned space-flight is now over and plans are well advanced for the exploitation era due to begin in 1980. NASA's Space Shuttle will reduce transportation costs and expand the potential applications of space technology by removing many of the constraints imposed by conventional launch vehicles. Spacelab is intended to capitalise on these principles.

A re-usable manned space laboratory with comparatively low operating costs for applications-oriented and scientific experiments, Spacelab is being developed by the European Space Agency (ESA) as a joint European endeavour and will be launched into near-earth orbit in the cargo bay of the Orbiter section of the Shuttle. Thus Europe is for the first time embarking on a new phase of space technology simultaneously with the United States.

The emphasis of the ESA's programme is on satellites for telecommunications, meteorology, Earth resources surveying and air and maritime traffic control. Scientific research is an important but by no means dominant component; the scientific satellite programme concentrates on upper atmosphere and magnetosphere research and astronomy, particularly in the X-ray and γ -ray regions. Spacelab complements these programmes to provide a balanced package of scientific research, but though maintaining the balance, not all future projects under consideration by ESA are likely to be funded. Areas of research will inevitably suffer as the emphasis shifts towards usage of major facilities, but the dwindling national programmes in Europe might be steered to help cover the gaps.

Where Spacelab fits

The Orbiter section of the Shuttle can be launched into near-Earth orbits of various inclinations with an altitude range of 200 to 900 km and a flight time of from 7 to 30 days. Equipment may be placed in orbit or returned for servicing and evaluation after the Orbiter has returned by landing like a conventional aeroplane. The Shuttle concept can be used for launching and servicing satellites, but the accessible range of orbits is limited and conventional 'throw away' launch vehicles will remain necessary unless the inter-orbit 'Space Tug' under consideration by NASA becomes a reality.

Spacelab is a particular unit that

slots into the cargo bay of the Orbiter. It is of modular design with two basic building blocks: a pressurised module providing a 'shirt-sleeved' environment for a manned laboratory connected to the living quarters of the Shuttle by a crawl-tube; and an unpressurised pallet on which instruments can be mounted and exposed directly to space. Various combinations of module and pallet are possible, providing a versatile manned space laboratory with a typical experiment payload of 5 tonnes. Up to four instrument operators can be carried (two are planned for the first flight); they will need a scientific background but do not have to submit to astronaut training—the standards required will be similar to those of commercial airline flight engineers.

The immediate advantages of this arrangement are that the stringent requirements on weight and volume that bedevil satellite experiments are relaxed, although the problems are to some extent transferred to the strict safety regulations necessary to protect the instrument operators. Existing requirements based on astronaut protection are unnecessarily severe for Spacelab, though some amelioration remains possible. Previous manned spaceflights which have shown the advantages of having operators for some classes of experiments have mainly involved man either as an experimental subject or in his capacity as a dexterous machine;

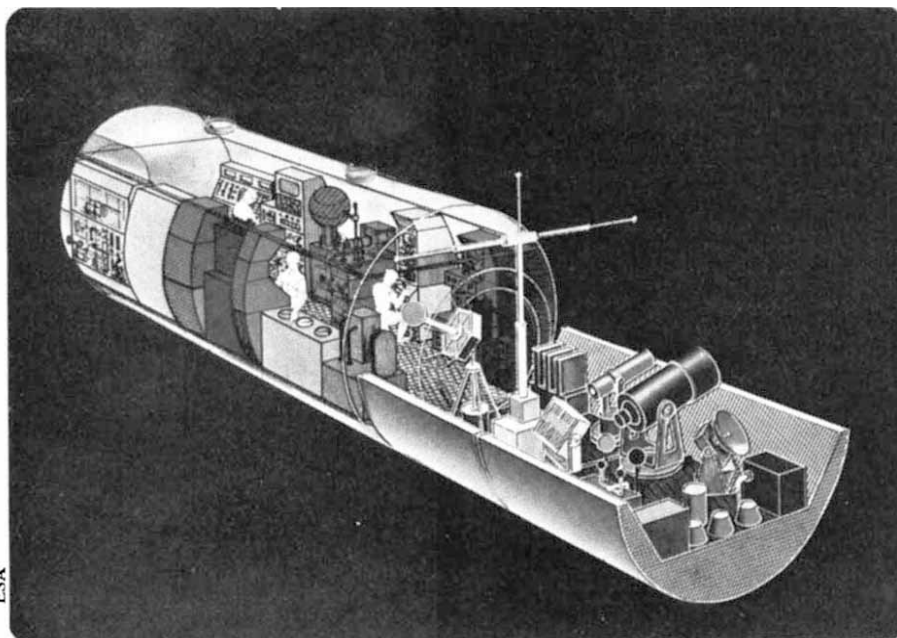
the decision-making capabilities of man in connection with an experiment have yet to be fully exploited. Re-usability is an important new facility: equipment can then be flown many times rather than abandoned at the end of each mission. Perhaps of greater significance is the ability to return materials processed or separated in a space environment and the means of collecting large volumes of data, stored for example on film, beyond the capability of feasible data transmission systems.

Cost immense

Clearly the cost of such an enterprise is immense; the design, development and delivery of one engineering model and one Spacelab flight unit is estimated at around \$500 million at mid-1975 prices. Currently the cost of the Spacelab project is under review and, according to ESA, "severe cuts in the deliverable hardware will be necessary in order to accomplish the development project within the established funding ceiling"—not an unusual problem in these times of high inflation.

Value for money is possible if frequent use is made of the facilities. Spacelab is intended to be carried on around 40% of the Shuttle flights and is designed for a minimum of 50 seven-day flights, or about 10 years of operation. This schedule allows the lead time for experiments to be much reduced from the present position with satellite projects. The advantages are clear; the realisation depends largely on funding and management.

Such a large international venture can easily become constipated by its own administration. The Spacelab concept can only be a success if flexibility is maintained for much longer than in



Spacelab: artist's impression

earlier space projects, but the organisational and management structure have at the same time to cope with an unprecedented mixture of political, financial, technical and scheduling constraints. Major efforts are being made to provide a structure capable of reacting sensibly to these problems.

Experience from previous manned space missions has been invaluable. It is generally agreed, for example, that insufficient thought and preparation went into many experiments conducted aboard Skylab, and a far greater degree of control is being exerted over Spacelab proposals. A simulated Spacelab mission was conducted in 1975 to test out the role of the instrument operators on board Spacelab and the suitability of the proposed management procedures. Short lead times for experiments were demonstrated to be practical, results being obtained from experiments executed only 16 months after proposals were requested, but the mission highlighted the need for greater integration of the instrument operators with the evolution of experiments, and a second more realistic simulation mission is planned for May 1977.

Spacelab research

Areas of research in which Spacelab will provide a valuable addition to satellite facilities include astronomy, atmospheric and solar physics and earth observation. Long continuous periods of observation are often required in these fields and Spacelab is seen as an intermediate step towards

manned orbital systems containing heavy and complicated astronomical equipment that would require regular maintenance; these 'Free-Flyers' would be serviced via the Shuttle.

The use of Spacelab simultaneously for astronomical and Earth observations clearly presents problems, and future Spacelab flights will be dedicated to specific areas of research. The flight profile is not really suited to investigation into the magnetosphere and certain atmospheric phenomena, and in these fields of research Spacelab will be used primarily as a satellite launcher, particularly as the Shuttle is liable to pollute the environment. Measurement of this pollution in terms of emitted gases and magnetic fields is one of the primary aims of the initial Spacelab flight.

For the life sciences and materials science Spacelab will provide a new opportunity for the study of fundamental problems in an environment unattainable on Earth. Additional benefits for the life sciences are the availability of a wide spectrum of high energy particle radiations, whereas materials science and plasma physics welcome the ready attainment of a vacuum over large volumes and the possibility of studying processes under containerless conditions.

The effects of weightlessness and radiation on biological systems are the major areas of interest in biomedicine on Spacelab, and the first payload will include a Space Sled facility for studying the effects of acceleration on astronauts under weightless conditions.

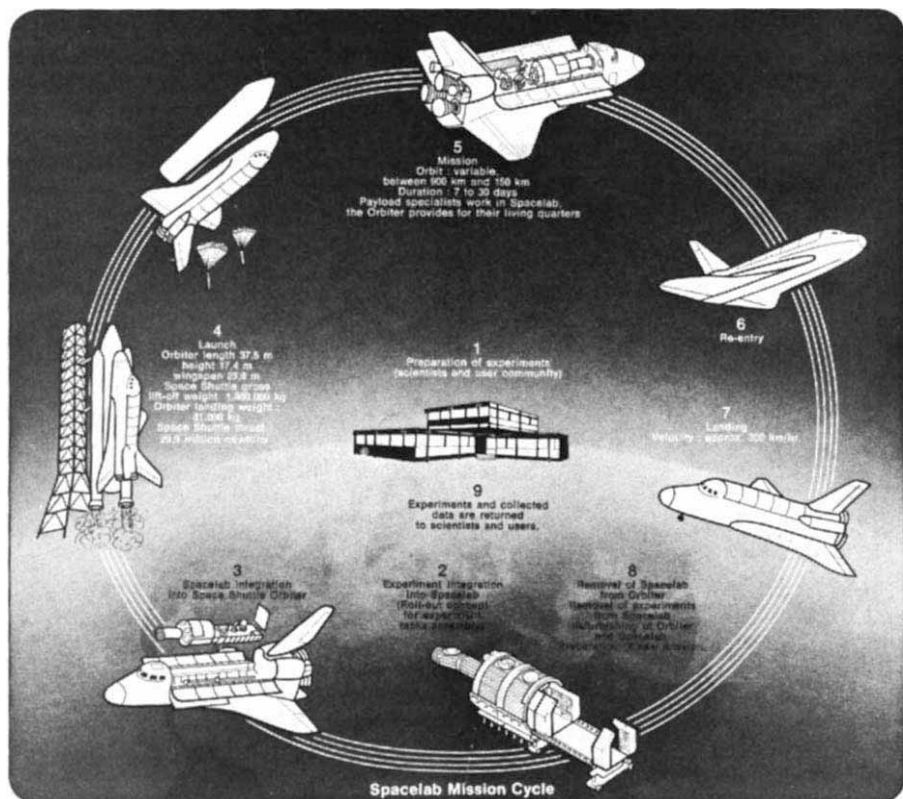
Materials science experiments require steady conditions which preclude the use of the Sled and spacecraft manoeuvres—luckily the majority of these experiments are of short duration.

Commercially the field of materials science offers immense potential and this is reflected in the set of experiments recently chosen as the payload for the first Spacelab flight where materials science experiments outnumber those in all other disciplines. The effective absence of gravity ($<10^{-1}g$) removes convection and buoyancy effects, so enabling fundamental physical and chemical processes to be studied in the absence of otherwise maskings phenomena and allowing the production of novel and improved materials.

In ESA jargon Spacelab is a 'Special Project'—a programme in which participation by all member states is not mandatory. Countries participating in the finance of the project get a proportionate share of the contracts and use of experimental facilities. The major contributors to Spacelab are Germany (53%), Italy (18%) and France (10%). The initial Spacelab flight in mid-1980 is a joint ESA/NASA mission in which the available facilities are shared equally between Europe and America. The primary objective of the first flight is to demonstrate the capability of Spacelab for space research and to check out all the support systems. Experiments therefore receive second priority, although 100 man-hours have been allocated to them and the necessary test programme restricts the power available to experiments to around 30% of that ultimately to be provided.

In spite of the limited experimental facilities available on the first test flight, the response to the call for experimental proposals has been high. Of over 2,000 applicants, a total of 222 have been accepted, providing 77 experiments from 16 countries. Some proposals were no doubt motivated by the feeling that previous Spacelab experience will carry weight in proposals for future flights; others sprang from uncertainties concerning the funding of these future flights, although these fears will be groundless if Spacelab can be used with its planned frequency.

NASA will charge around \$10 million to launch each subsequent Spacelab mission. Details of how this cost will be distributed between participating states within ESA and the experimenters have yet to be worked out. This cost is small compared with the investment, and it will be a pity if details over funding jeopardise the exploitation of Spacelab. It will be a disaster if ESA's future proposals for a balanced scientific programme are affected. □



USSR

● The need for a closer connection between research and the requirements of the national economy is a constant theme in Soviet planning. Last autumn, the Far Eastern Science Centre was criticised by the Party for having failed to direct its research towards local needs. More recently, the prestigious Siberian Branch of the Academy of Sciences of the USSR came under similar censure. A statement from the Central Committee of the Party, while praising the Siberian Branch for its work in fundamental research, finds "insufficiencies and unresolved problems" in its activity; work on "the complex use of the natural wealth of the eastern regions of the country" is going too slowly; the Presidium of the Branch is paying too little attention to the work of certain centres; and filials, scientific forces and material resources are not being concentrated on "the most important lines of science connected with the acceleration of scientific and technical progress". In particular, insufficient attention has been paid to the problems of the Kansk-Achinsk coal basin, the Norilsk mining and metallurgical combine, and the route of the Baikal-Amur Mainline (BAM) railway.

Instead of admitting these faults and promising amendment, however, Academician Gurii Marchuk, the Chairman of the Siberian Branch, issued a statement extolling the work of the Branch, and softening the rebuke of the Central Committee to a gentle directive showing "ways of improving the quality and efficiency of scientific research". Marchuk stressed that during the 20 years of the Branch's existence, science centres, in addition to its main centre at Novosibirsk, have been established in many cities including Irkutsk, Ulan-Ude, and Krasnoyarsk. The Siberian Branch now includes 48 research and design organisations, representing practically every field of the natural and social sciences. Marchuk himself envisaged the future of Siberian science as "a growing effective force, transforming huge tracts of land along the Leninist path and exerting an increasing influence on many sides of the life of Soviet society".

Within a week, the critical tone of the Central Committee had been de-emphasised even further. Addressing the Moscow meeting of the heads of Academies of Science of the Comecon block, Mr Brezhnev held up the Siberian Branch and the Ukrainian

Academy as examples for emulation. "The Siberian Branch is carrying out a very interesting experiment", he said. "The Institutes of this Branch are directly connected to many sections of the national economy, with many major [industrial] enterprises. They have worked out long-term programmes of scientific-technical cooperation and are gradually implementing them."



Since the Brezhnev speech, articles have appeared in the Central press commending the work of individual Institutes of the Branch in the service of the economy—the Hydrodynamics Institute, has been commended for surveying the line for the BAM and the Institute of Atmospheric Optics for weather-forecasting. Marchuk's defence of his Branch seems to have been successful and its present shortcomings have been accepted as temporary deficiencies rather than a failure to understand the fundamental task of the Branch.

● Although the Soviet lunar research programme has so far been confined to unmanned probes and lunokhod rovers, biophysicists at the Siberian Branch of the Academy of Sciences are already working on the possibility of farming in lunar conditions. Experiments with wheat, carrot, beetroot and turnip seedlings suggest that plants could be grown under suitable transparent domes using the natural lunar illumination cycle. The plants were kept at a temperature of 3–4 °C during the 'night' periods, and were grown hydroponically, using common tap water as the nutrient medium. In spite of the lack of minerals, especially nitrogen, and the lunar illumination cycle, all the plants produced roots with the "normal biochemical composition", and during the second lunar 'day' the wheat produced ears. The team stresses that if plants can be grown

successfully by natural sunlight, the energy problem of settlements on the moon would be much easier.

● The current difficulties with international fishing limits have evoked considerable rethinking of Soviet fisheries policy; in contrast to the old theory of large catches and the over-fulfilment of targets, the new line stresses that since catches are limited, the entire quota must be suitable for food. The new policy is to be implemented in various ways: Latvia is to use refrigerated factory ships in the Baltic to reduce wastage; and the Deep Sea Fishing Flotilla, operating in the Sea of Okhotsk, has been instructed not to catch Okhotsk grenadier (which accounts for up to 50% of all catches in the area) since the experts have decided that grenadier is unmarketable.

Following a recent order from the Ministry of Fisheries, likely lakes and reservoirs for fish-farming are being appraised, and existing fish-farms are being extended. An experimental trout-farm is already in operation in the out-fall of the Kola atomic power station in the Arctic. The use of fish wastes is also being investigated. According to an official of the Sevryba (Northern Fisheries) organisation, the fishing industry has for some time been able to collect and deliver heads, tails, and gut to shore enterprises for further processing. These waste products, he stressed, contain 30% fat, 8–12% protein and a "considerable quantity" of the B vitamins. At present such wastes are used for animal food, but research is underway on their possible use for human consumption—a project more likely to appeal to the planners than to the average consumer.

● The RATAN-600 radio-telescope, the largest in the world, is at last officially in operation. The telescope, sited at Zelenchuk in the Stavropol' district, was constructed jointly by the Academy of Sciences of the USSR, Moscow University, the Ministry of Power and Electrification, and the Ministry of Power Plant Construction. Mr Brezhnev's formal message of congratulation was issued on 20 March, 1977.

Each of the four sections of the telescope can operate autonomously and part of the Ratan-600 has been receiving signals since 1974. A number of research projects are already in hand including studies of the fine radio-structure of the sun, the moons of Jupiter, and the galactic nucleus.

Vera Rich

correspondence

Travel rights

SIR,—I was very sorry to hear of the incident involving Academician Malek's passport reported by Frantisek Janouch (6 January, page 10). Ivan Malek has been a personal friend of mine for many years and I have learned to respect his wise and humane views. Personally I feel it is a tragedy that present circumstances make it very difficult for him to play the part in building up a strong, forward looking and socially concerned science in his country for which his talents so clearly qualify him.

There can be no doubt about the attitude of the World Federation of Scientific Workers toward the travel rights of scientists. I am sure Dr Janouch knows that ever since its foundation 30 years ago our Federation has worked actively for the maximum level of contacts between scientists in different countries. We fully support the UNESCO Recommendation on the Status of Scientific Researchers (1974), Article 26 of which says:

Member states should actively promote the interplay of ideas and information among scientific researchers throughout the world, which is vital to the healthy development of science and technology; and to this end should take all measures necessary to ensure that scientific researchers are enabled, throughout their careers, to participate in international scientific and technological gatherings and to travel abroad.

Following the decision of our 11th General Assembly held in London last September, the WFSW set up a sub-committee to make these recommendations more widely known and to work actively for their practical implementation. If Dr Janouch will supply detailed, and if possible, documentary information about the incident to which he refers I am sure our sub-committee will examine it and suggest what appropriate steps, if any, the Federation should take.

E. H. S. BURHOP

World Federation of Scientific
Workers,
London, UK

Toxic levels

SIR,—It is not clear what are the areas of expertise of your correspondent Thomas H. Jukes (10 February, page 491). It is clear however, that one of his areas of ignorance is chemical carcinogenesis.

How does he know that "substances that are toxic at high doses are

sufficiently harmless in the amounts actually present that they offer no finite hazard to health"? This is the key to the argument about carcinogenic chemicals in the environment. They give rise to tumours in significant number when tested in small groups of experimental animals for a maximum of two years. How do we extrapolate such findings to tens of millions of people whose susceptibility is unknown but might have a wide range, both above and below that of our experimental animals, and who live for 60 or 70 years?

If we reduce the doses given to our experimental animals we reach a dose at which one or two animals in a group of 100, say, will have a tumour not seen in a group of 100 untreated controls. Although this is 1 or 2% of our population of animals (equal to 2 or 4 million of the US population), we would say that this incidence of tumours is not statistically significant, and some would claim that this dose is 'safe'. I would not. In practice, we need an incidence of tumours exceeding 5 out of 100 to be sure of statistical significance. So, because the effects of carcinogens are cumulative, we cannot establish a safe dose for any carcinogen, because we don't know how. Similarly, in our animal experiments in which we usually test single compounds we cannot take account of the probable additive or synergistic effects of several carcinogens at low doses, as we must experience them in daily life. That is why we must be wary of any carcinogen when we discover it at any level, even at the risk of appearing to be lunatic consumerists.

Perhaps Jukes should ponder the fact that there is a lot of cancer around and hundreds of thousands of people die of cancer every year, almost always of unknown cause. It is my contention that part of this cause is the carcinogenesis test which chemical manufacturers and food processors, together with Nature herself, are conducting in us, presumably with Jukes' approval.

WILLIAM LIJINSKY

Frederick Cancer Research Center,
USA

Thomas Jukes replies: Lijinsky misquotes me: my statement was "... food contains substances that are toxic at high levels but are sufficiently harmless in the amounts actually present that they offer no finite hazard to health".

With this misquotation corrected, the basis for his peroration disappears. My statement was essentially a reference to the basic principle of toxicology first enunciated by Paracelsus¹.

There are numerous examples of such substances, such as solanine (potatoes), cyanide (lima beans), goitrogens (cabbage), estrogens (soybeans, wheat germ), and selenium (marine fish). I am glad that Lijinsky included Nature in his list of providers of carcinogens. His discussion of the "safe dosage" of carcinogens alleges that "some would claim that this dose" (that is a dose that would produce tumours in "2 or 4 million of the US population") "is safe". I can think of no one who would make such a claim; certainly I would not. Various authors, including myself, have discussed the problem of reaching adequately low levels of dietary carcinogens²⁻⁶.

It is certainly possible to postulate levels that pose a risk of less than one case per year in the US population, or that will not be effective unless we live for more than 200 years. However, it is not possible to devise a diet of natural foods that is free of "carcinogens", no matter whether they are "additive or synergistic" (as Lijinsky says), or even antagonistic (which he does not mention).

In his final sentence, Lijinsky incorrectly assumes that I approve of carcinogenic food additives. I favour bans on Reds No. 2 and No. 4, but I pointed out that many consumers apparently wanted to go on eating red maraschino cherries despite the findings on the dye. Many also seem to want cyclamates, saccharin and cigarettes. The current protest by consumers against banning saccharin is an effective but an unexpected support for the point I made. Consumerists wish to decide which carcinogens should be banned. Lijinsky often warns against bacon, but not against barbecuing of steaks, despite his own demonstration that this produces carcinogens⁶, and not against tobacco, which smoked or chewed supplies nitrosamines.

A list of my publications related to cancer is being forwarded to Lijinsky.

¹ Paracelsus, *Drey Bücher Heils of Arnold Byrckmann*, Cologne, 1564.

² Jones, H. B., and Grendon, A., *Food and Cosmetic Toxicology*, 13, 251 (1975).

³ Jukes, T. H. *Preventive Medicine* 5, 438, 1976.

⁴ Claus, G., Krisko, I., and Bolander, K. *Food and Cosmetic Toxicology*, 12, 737, 1975.

⁵ Druckrey, H., *Potential Carcinogenic Hazards from Drugs, Evaluation of Risks*, 7, 60, Springer Verlag, Berlin 1967.

⁶ Lijinsky, W. & Shubik, P. *Science* 145, 53 1964.

news and views

Antibody production made easier

from K. I. Welsh

THE latest paper from the laboratory of César Milstein (this issue of *Nature* page 550) reporting the construction by cell fusion techniques of a line of hybrid cells secreting antibody against rat major histocompatibility antigens, promises to be an important step forward in tackling some of the many clinical and biological problems concerned with the histocompatibility system which require a reliable and standard supply of anti-histocompatibility antigen antibodies for their solution.

Tissue matching for skin, cornea, bone marrow and kidney transplants depends to a large extent on stocks of reasonable titre monospecific typing antibodies against the major histocompatibility antigens. These precious sera, obtained from immunised volunteers, multiparous women, transplant recipients or primates take months or even years of laborious work to produce, test and become accepted as standard reagents. Antibodies, over 200 in all, are tested for lysis of recipient cells and potential donor cells; identical reaction patterns indicate identical recipient and donor histocompatibility type at the HLA-A, B, C and D locus. Commercial reagents are expensive, yet the use of antisera against histocompatibility products in the analysis of the association of particular diseases with the major histocompatibility complex (MHC) as well as for routine tissue matching, is increasing daily. The ideal solution to these serological problems would be for sufficient supplies of standard typing reagents to be available worldwide.

The isolation and characterisation of MHC products on a large scale and experiments designed to answer many basic questions related to the understanding of their evolutionary significance, present role and importance in tissue rejection and prevention of disease would also be made much easier

with a readily available supply of standard monoclonal antibodies to the MHC antigens.

Milstein *et al.* constructed their histocompatibility antibody secreting cell line by fusing a mouse myeloma cell line with spleen cells of AO-strain rats previously immunised against DA strain antigens. Clones grown up from individual hybridisations were tested for their ability to produce anti-DA antibodies, and a clone was selected which was further characterised as producing antibody against an individual K/D specificity of the Ag-B4 haplotype, thereby identifying the first uniquely defined antigenic specificity in the rat MHC. The authors hope that characterisation of the products of other clones will lead to the identification of other specificities of the Ag-B4 haplotype.

This work illustrates the latest use of basic techniques developed in Milstein's laboratory for producing hybrid cell lines secreting antibody of defined specificity (see Köhler & Milstein *Nature* **256**, 495; 1975), a technique now being exploited in other laboratories.

At a recent discussion meeting at Cambridge K. Rajewsky and G. Hämmerling reported that they have hybridised tumour cells with a variety of antibody-producing cells to obtain lines of cells synthesising antibody against a wide range of idiotypes as well as bacterial and mammalian cell components.

Antibody production from tissue cultures of such cells requires culture facilities on a larger scale than many laboratories possess. Rajewsky *et al.* have however developed an *in vivo* culture system which brings production of experimental quantities of antibodies from hybrid cells easily within the capabilities of most laboratories. The hybrid cells are injected into a histocompatible animal where they pro-

liferate, producing high levels of antibody which can be recovered from the animal's serum. This provides a method of obtaining experimental quantities of antibody from many fewer animals than needed with techniques now used.

At present, 100 rats are needed to produce 10^5 lytic units of anti-Ag-B serum for example, but using the Milstein/Rajewsky approach only one rat would be needed, although perhaps 10 rats would be necessary to cover all the variants of specificity.

The commercial importance of such work is that it converts antibody production from a biological science with all its inherent variability and non-reproducibility into the realms of synthetic chemistry where any desired reagent is available off the shelf and can be assumed with reasonable confidence to be identical to the same reagent in any other laboratory. Monoclonal antibodies do have disadvantages; for example, a monoclonal anti-HLA-B7 reagent might only react with 10% of the HLA-B7 individuals defined by a polyclonal serum. Such disadvantages can be easily circumvented however, and there will be the advantage that it will be possible to identify the variants of histocompatibility specificities such as HLA-B7 so precisely that near perfect tissue matchings can be made, and the precise relationship of disease to the HLA-B7 complex defined.

The potential of cell fusion in immunology extends far beyond immunoglobulin production. It should eventually be possible to produce a range of helper, suppressor and factor-producing cell lines. The development of the Milstein technique now offers the British government a unique opportunity of extending this work by financing a centre for the production of such hybrid cell lines and for UK companies to take the commercial initiative. □

Neuroanatomy of visual acuity

from Peter Lennie

ONE of the aims of vision research is to find the structures that underly our visual capabilities; sometimes the relation between structure and function is striking. There is a regular correspondence between points on the retina and points on the primary visual cortex in man, but because the cortical projection of the fovea (the area of the retina responsible for the resolution of fine detail) is huge in relation to the projection of the peripheral retina, the map on the cortex is a distorted representation of the retinal surface. The large projection of the foveal region seems to be required for the resolution of fine detail, for there is a close relation between the "cortical magnification factor" (M)—the distance in mm on the visual cortex corresponding to an angle of one degree subtended at the eye—and visual acuity, expressed as the angle subtended at the eye by the smallest object that can be resolved. Cowey and Rolls (*Expl Brain Res.* **21**, 447; 1974) showed a high correlation between $1/M$ and the minimum angle of resolution (MAR) at different points in the visual field, which suggests that a constant amount of visual cortex is devoted to the minimum angle of resolution, whatever its value.

The much magnified cortical representation of the foveal region may originate in the distribution across the retina of the ganglion cells whose axons project to the lateral geniculate nucleus (LGN), which relays to the cortex. With the exception of the foveal region the density of ganglion cells decreases regularly with increasing distance from the fovea, and is found to be proportional to M^2 . Thus over most of the visual field the magnification factor could be explained by assuming that, on average, each ganglion cell projects to an equal area of visual cortex. In the foveal region, where acuity is best and cortical magnification highest, the relationship between ganglion cell density and M^2 breaks down however, probably because the ganglion cells are arranged in a band around the foveal cones to which they are connected and thus are displaced from their receptive fields.

If the displacement of ganglion cell bodies causes the breakdown of the proportionality between ganglion cell density and M^2 , an allowance for this displacement should extend the proportionality into the very centre of the fovea. Rolls and Cowey (*Expl Brain Res.* **10**, 298; 1970) have done this for monkeys and Drasdo (this issue of *Nature*, page 554 has now made a

correction for ganglion cell displacement in man, by computing the density of ganglion cell receptive fields at different distances from the fovea. He has shown an impressive correlation between calculated receptive field density and M^2 from the fovea to points 40° in the peripheral retina, and has also formulated an equation for which he claims some generality in predicting visual performance at any point in the visual field.

These findings suggest a remarkable simplicity in the arrangement of the mechanism subserving visual acuity. However, other recent work has revealed new complexities in the structure and function of the visual system that make these simple relations rather surprising.

One of these is that retinal ganglion cells project to several areas of the brain (notably to the superior colliculus) besides the visual cortex. We cannot be clear therefore about the significance of the simple relations between visual acuity, cortical magnification and the overall density of ganglion cells until we know what fraction of cells projects to the visual cortex, and whether this fraction varies with position on the retina. The answer to this question is unclear, although recent evidence (Bunt *et al.* *J. comp. Neurol.* **164**, 265; 1975) suggests that in fact virtually all ganglion cells project to the cortex through the LGN and therefore that projections to other areas arise through collateral axons from the same cells.

A more intriguing problem arises from the fact that there are several different types of ganglion cell, distinguishable histologically (and in the monkey also physiologically), and so we also need to know how the different cell types contribute to the population of cells that does project to the cortex. Although it is now more than 30 years since Polyak gave a thorough anatomical description of different cell types in the primate retina, only recently has much attention been given to their possible functional roles. The impetus to much of this work was the discovery in the cat's retina of several classes of ganglion cell that are physiologically and morphologically distinctive and in some cases at least have different projections in the brain (see Levick in *Nature* **254**, 659; 1975 for a review). The primate visual system also seems to contain ganglion cell types that are physiologically distinctive (De Monasterio and Gouras *J. Physiol. Lond.* **251**, 167; 1975), although these seem to be

less clearly differentiated and are not so obviously related to morphology. One is therefore led to ask what different functions they subserve and whether their projections on the primary visual cortex are topographically different.

Since the physiologically different cell types are distributed differently on the retina it seems likely that they will project differently on the primary visual cortex. This being the case it is puzzling that M , which must reflect the projections of all classes of ganglion cell, should be so simply related to MAR, which one supposes might be mediated by a single cell type. Perhaps this class is predominant, and cells of other types, even if they differ in their distribution on the retina and their projections on the cortex, occur in numbers too small to upset the simple relation between cortical magnification and minimum angle of resolution.

We might obtain some clearer idea of the functions of the putative minor classes of cell if a variety of tasks were used to examine different forms of visual acuity in relation to position in the visual field. At all events, the heterogeneity of ganglion cells argues for caution in interpreting relations between the gross structure and function of the visual pathway. □

Historical ichthyology

from a Correspondent

TAXONOMISTS are forced by the disciplines of their calling to be continually aware of the past history of their field of study. At a basic level they are obliged to refer to the earliest description or published name for an animal or plant, and possibly more than in most sciences the constant reference back to the early literature is part of the taxonomist's way of life; to a large extent the early literature is as significant as later syntheses. In this context it is not surprising that traditional taxonomists are often deeply involved with the history of their field, or with the life and work of earlier students of their subject. A recently published study of Francis Day (1829–1889) and his collections of Indian fishes by P. J. P. Whitehead and P. K. Talwar (*Bull. Br. Mus. nat. Hist. (Hist. Ser.)* **5**, 1; 1976) is an excellent example

of such painstaking research.

Francis Day was born in 1829 in Sussex, and qualified in medicine at St George's Hospital in London (1851). Immediately after qualifying he joined the Indian army as Assistant Surgeon and between 1852 and 1863 worked in the medical service in India, broken only by a year's sick leave in England in 1857. Until about 1863 his career was largely medical but then he increasingly became involved in natural history studies especially those of fishes. During 1864 and 1865 he was in England, presenting his first major paper, *On the fishes of Cochín* (*Proc. zool. Soc. Lond.* 2-40, 286; 1865), and late in 1865 publishing the *Fishes of Malabar* (Quaritch, London) a major work on Indian fishes. In 1866 he returned to India accompanying a consignment of trout eggs in an (eventually unsuccessful) attempt to establish this fish in the Neilgherry Hills. From then until 1869, although nominally still employed in the medical service of the army, Day became increasingly involved in fishery surveys and the study of fishes. In 1871 he was appointed Inspector-General of Fisheries, and commenced a series of active surveys in various parts of India. In 1874 he returned to England to begin writing *The Fishes of India* (published in four parts between 1875 and 1878, with a supplement in 1888). This book is still the only reference work on the fishes of the subcontinent as a whole, and, although many revisionary studies of groups of Asiatic fishes have been published since, reference to *The Fishes of India* is still obligatory for the student of Indian fishes.

In his later years Francis Day turned his attention to European fishes, and his *Fishes of Great Britain and Ireland* (1880-1884) and *British and Irish Salmonidae* (1887) were for many years classics in their field. They are now highly prized by the antiquarian book trade, as are his other books, which perhaps still indicates their value. Francis Day died in 1889 at Cheltenham, Gloucestershire.

These are the bare bones of Francis Day's career as surgeon and ichthyologist, and they correctly suggest a man of remarkable energy and achievement. Whitehead and Talwar provide far more detail and have produced a rounded study of the man (despite their modest statement that there is "As yet, . . . no full biography of Francis Day"), and the difficulties that he had to face with unsympathetic superiors in India, and the criticisms and obstructiveness of Albert Günther, the British Museum zoologist. Their assessment of Francis Day is that he "was one of the outstanding figures in that phase of ichthyology which called

for comprehensive works on the fishes of particular regions".

Beyond the themes of biography and assessment of Day's work, Whitehead and Talwar concentrate on the distribution of Day's superlative collection of fishes. Although his collection was brought to England when he was writing *The Fishes of India*, less than half of it found its way to the British Museum. No fewer than fourteen museums received parts of his collection either by gift or sale, and his personal library and many manuscripts passed to his daughter and thence to Cheltenham Public Library. Major collections were sent to the Indian Museum, Calcutta, the Australian Museum, Sydney, the Naturhistorisches Museum, Vienna, and the British Museum (Natural History). These four collections between them hold most of the type material of species described by Day, and Whitehead and Talwar present a tabulation of all the syntypic material of Day's species in eleven museum collections. Their painstaking listing of extant specimens and the route which each lot followed to the appropriate museum, with Day's indication of their importance, is a major contribution for later taxonomists working on Indian fishes.

That so little of Day's collection (and that, in general, the less important material) was to be found in the Natural History Departments of the British Museum, later (1880) to become the British Museum (Natural History), was due to the acrimonious relationship between Day and Albert Günther. Whitehead and Talwar deal with the beginnings of this bitter episode and its continuance until just before Day's death. The quarrel has also been touched upon recently by Günther's grandson, A. E. Günther (*A Century of Zoology at the British Museum . . .*, Dawsons, London; 1975), but now that both sides of the story can be compared it is clear that Günther was particularly unjust in his relationship with Day. The immediate result was that the most important portion of Day's collection was sent abroad and not, as would have been expected at that time, lodged in London.

Although Whitehead and Talwar recount this episode of nineteenth century zoological in-fighting in detail, and one must admit it makes fascinating reading, their study of the history of Day's collection is a fundamentally important contribution to furthering the study of the taxonomy of Indian fishes. □

Polyphosphazenes: tailored polymers

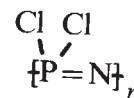
from Paul Calvert

THE polyphosphazenes are a versatile group of polymers based on an alternating phosphorus and nitrogen chain, which are potentially useful as low temperature, solvent resistant elastomers and may also have a number of applications in medicine. Recently published calculations of conformational energies shed light on the origin of their rubber-like behaviour and nicely illustrate both the gap between calculable and observable quantities and the benefits which could be gained by closing the gap.

Inorganic polymers have been a subject of interest for many years, partly in the hope of finding materials which would be resistant to temperatures above 350 °C where most organic polymers start to oxidise and partly, one suspects, to allow inorganic chemists to make themselves useful. The effort has not been a great success, to a large extent because many of the polymers produced are thermally stable but very vulnerable to hydrolysis. The only important group of commercial inorganic polymers is the silicone rubbers which are used as low temperature, chemical

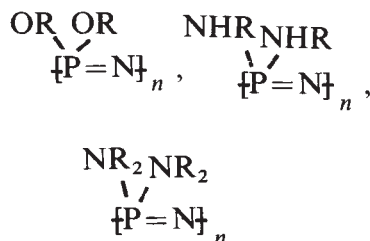
and solvent resistant elastomers (glass transition temperature 150 K). Their main drawback is that they are mechanically weak.

The polyphosphazenes have the greatest promise of the remaining inorganic polymers. The simplest example is poly(dichlorophosphazene) produced



by polymerisation of the trimer hexachlorocyclophosphazene which is the product of reaction between ammonium chloride and phosphorus pentachloride. This polymer readily hydrolyses starting at the chlorine-phosphorus bond but the trick to producing stable polymers found by Allcock and coworkers in 1965 is to replace the chlorine with organic groups linked to the chain by either an oxygen or nitrogen atom. This is done by reacting the chlorinated

polymer directly with the appropriate amine or sodium alkoxide.



Since then Allcock's group at Pennsylvania State University have published about 30 papers describing the synthesis and properties of a large number of these polymers (see *Science* **193**, 1214, 1976; and *Chemtech* 553, September 1975). The interest of these polymers lies in this versatility, an indefinite number of side groups can easily be added to produce a wide range of properties without having to perform a new polymerisation each time. The low temperature flexibility of some of these materials can also be applied.

Low temperature elasticity is necessary not only to make rubber grommets for use in cold climates but also in the pumping and handling of cold liquids. A necessary condition for this is believed to be chain flexibility, that is, unhindered rotation about the chain backbone bonds. In the polyphosphazenes and silicone rubbers this arises by the alteration of an unsubstituted chain atom (the nitrogen) with one carrying only small substitutes (the phosphorus) so that internal rotation can occur without the side groups colliding. Allcock, Allen and Meister (*Macromolecules* **9**, 950, 956; 1976) have made a start toward quantifying this by calculating the potential energy as a function of conformation for a series of halo- and organo-substituted polyphosphazenes. These calculations assume a Lennard-Jones interatomic potential and then evaluate the potential energy due to neighbouring groups on the chain as a function of the bond angles. However the chain does have a conjugated double bond structure which might also hinder rotation, although Allcock asserts that it does not on the basis that the orbitals will be symmetric about the bond axis. The exact nature of the P-N double bond is still a matter for debate and they may have to be taken into account (Allcock *Phosphorus-Nitrogen Compounds* Academic, London-New York, 1972). The potential energy barrier to bond rotation calculated in this way is directly related to the chain flexibility and can be compared with the polymer glass transition temperature which varies from 180 K for the difluoro polymer to 265 K for poly(diphenoxyposphazene). The calculated barrier heights correlate

Ultraviolet windows in sunglasses

from John Walker

CAN sunglasses damage the eyes? Two American scientists have discovered that many sunglasses on sale in the United States are not effective in filtering out ultraviolet radiation; they may actually increase the possibility of eye damage in bright sunlight.

Visible light has wavelengths between 400 nm (blue/violet) and 700 nm (Fig. 1). Other wavelengths, which are at best unnecessary for vision and at worst a danger to the eyes, ought to be removed by sunglasses before reaching the eyes. Ultraviolet (300-400 nm) in particular is suspected of causing cataracts.

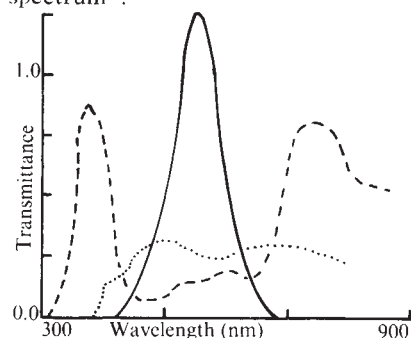
Anderson and Gebel (*Appl. Optics* **16**, 515; 1977) tested sunglasses they had randomly purchased in local shops and drugstores (in Ohio, USA). Many of them were optically unsatisfactory and "... seem to be designed more to satisfy the fashion whims of the wearer than to shield the eyes from unnecessary radiation". One-third of their selection transmitted more light in the ultraviolet than in the visible—in the worst case 70% transmission at 360 nm compared with 20% at 550 nm (Fig. 1). This may be dangerous for two reasons.

First, as the amount of visible light reaching the eye is cut down, much stronger outside light levels can be tolerated without discomfort, and so the ultraviolet exposure may be greater than if no sunglasses were worn. Alternatively, the reduced visible light intensity causes the pupil to dilate allowing more light to enter the eye. Anderson and Gebel calculate that the worst sunglasses

actually double the amount of ultraviolet absorbed by the eye (Fig. 1); thus there is a greater risk of eye damage than if no sunglasses were worn.

Some sunglasses were quite satisfactory, however. Figure 1 shows the transmission spectrum of one of the better pairs—though even there it would be better if the transmission cut off at 450 nm instead of 390 nm. And unfortunately there appeared to be no correlation between ultraviolet performance, price, lens colour or manufacturer; there is no way of picking a good pair other than by measuring its transmission spectrum.

The authors conclude that their preliminary study should be extended, and should include prescription sunglasses (the only pair they tested performed very well). Furthermore "... it would be in the consumers' interest for manufacturers to indicate in some manner the transmission characteristics of their lenses over the solar spectrum".



The relative eye sensitivity (full line), and optical transmission curves for the worst (dashes) and best (dots) pairs of sunglasses.

well with the observed glass transition temperatures for the halo- and organo-substituted polymers separately but poorly when the two groups are compared, the organo compounds having larger barriers to rotation for similar glass transition temperatures. Clearly some other factor must be taken into account.

This question should really be taken further as there has been relatively little advance since the theory of Gibbs and DiMarzio in 1962 stressed the importance of chain flexibility for the glass transition. The polyphosphazenes provide a good system on which to relate properly the calculated conformational energies to the glass transition temperatures since the ease with which substituents can be changed on the same backbone should allow the isolation of factors such as polarity,

side group flexibility and copolymer composition.

The proposed uses for the polyphosphazenes also reflect the effect of side groups on the polymer properties. Small side groups give a low glass transition temperature and resistance to swelling by hydrocarbons, desirable for gaskets and O-rings. Crystallisation is prevented by the use of mixed substituents so that the polymer is rubbery. With some subtlety it should be possible to produce a rubber which crystallises only on stretching. This would give good strength properties comparable to natural rubber rather than to silicone rubber which cannot crystallise and is weak. By adding fluorinated substituents polymers can be produced with a low surface energy which could be used for water repellent coatings and for surgical implants

where a low surface energy is believed to reduce the tendency of implants to cause blood clotting. The amino-substituted polymers can be made water soluble and are susceptible to hydrolysis to varying extents. Uses as resorbable sutures and slow release drug carriers are being investigated.

There are a number of groups of polymers where a wide range of properties can be produced by altering the side groups. The proteins and polysaccharides are two classes which have proved their value over the years. Vinyl polymers which range from polyethylene to polyacrylamide could also be seen as such a class. Polyphosphazenes are unlikely ever to be used on a large scale but they do seem to have promise in terms of tailoring polymers for specific, small scale uses. Here the ability to add substituents without having to perform a new polymerisation is a real advantage. This approach of designing polymers for specific purposes would be real molecular engineering, something that is more often talked about than practised. The snag is that our current methods of predicting properties such as solubilities and transition temperatures are so crude as to shame most other branches of engineering. □

Neural systems

by Miranda Robertson

The Dahlem Conference on the Formation and Function of Neural Systems was held in Berlin on 7-11 March, 1977. The proceedings will be published and can be obtained from Dahlem Konferenzen, Delbrückstrasse 4c, D-1000 Berlin 33.

ALTHOUGH nominally the meeting was addressed to the function as well as the formation of neural systems, it was effectively dominated by the question of formation.

The growing contribution of genetics to the issue was divided by Peter Lawrence (MRC Cambridge) into two categories: the identification of genes which control development directly, and the identification of crucial cellular interactions through neurological mutants in which they have gone astray.

Two recent advances discussed at the meeting fall into the first category. One is the addition of isogenic locusts to the menagerie of mutants, mosaics and isogenic invertebrates on which much neurodevelopmental insight depends. A series of isogenic strains has been used by C. Goodman (in experiments described by D. R. Bentley, University of California) to distinguish genetically controlled variation from

Parental care

from John Krebs

ONE characteristic of higher vertebrates is that they usually look after their eggs or young, a trait which is rather rare in fish, amphibians, and reptiles. The advantages of parental care in terms of survival of young may be obvious, but it is less clear why one sex rather than the other should provide the care. In birds and mammals the trend is for females to do more than males for their young, while in fish it is often the other way round.

The theory of natural selection leads one to expect that each parent should, at any time during its reproductive life, adopt the course of action which maximises the number of young produced in the remainder of its career. (It should not, of course, make the Concorde Fallacy of directing its future action on the basis of past investment (Dawkins & Carlisle *Nature* **262**, 131; 1976)). For example, if the survival of young is greatly enhanced by care from one parent, but the second parent makes rather little difference, it would pay each parent to try and leave the young in the care of its partner, to go off and mate again. On the other hand if the survival of young depends (as in most birds) on care from two parents, it will pay neither to desert.

An analysis of the theory of parental care and desertion has recently been published by J. Maynard Smith (*Anim. Behav.* **25**, 1; 1977). He considers two situations in which breeding is synchronised and seasonal: in one the factor-limiting production of young is parental care, whereas in the other, the number of eggs laid by the female is also important. In the first model, it would not pay the female to produce extra eggs since they would not survive without additional care. The first model applies to most birds. Birds are usually monogamous and both parents care for the young, but if one parent can look after the young (as in species with precocial young such as gallinaceous

birds) and there is a reasonable chance of a second mating, it is usually the male which deserts. Although it would equally pay the female to desert, she is subject to a time constraint: she has to wait after copulation until she has laid the eggs, and this may mean that she is too late to have a chance of finding a second mate when there is synchronised seasonal breeding. The female can however encourage male fidelity by delaying tactics. If she refuses to copulate until the male has little chance of finding a second unmated female, she may force him into faithful monogamy.

In most fish, and a few birds, parental care takes the form of guarding and not feeding, so that the number of young reared by a female will depend not solely on the amount of parental care, but also on the initial production of eggs. Here the female has, so to speak, a choice of directing her reserves into parental care and laying fewer eggs, or channelling all her reserves into eggs and deserting each clutch. As long as one parent can rear the young, it will pay the female to desert, which she often does. As pointed out by Dawkins and Carlisle (*op. cit.*), an additional factor favouring male parental care in fish is external fertilisation. With external fertilisation the male has to deposit his sperm after the eggs have been laid; as a result the female has the chance to desert first, just as in species with internal fertilisation the male has first chance.

Mammals are rarely monogamous; male desertion is common because of the physiological commitment of the female to parental care, at least in the early stages. There are, however, some monogamous mammals, such as the gibbons (*Hylobates* spp.), and it is perhaps somewhat surprising that these species have not evolved more male parental care, for example in the form of male lactation. □

epigenetic 'noise' in the determination of an identified neurone. While the position of the cell body varied only between strains (implying genetic control), the terminal branching of the axon varied between three positions, at different axonal lengths, within strains (implying epigenetic control).

Deducing the nature of the genetic control from mutants is, according to Lawrence, enormously complicated by the fact that most developmental mutations are bound to be lethal,

which means that survivors are probably 'leaky' mutants and the investigator is thus faced with deducing wild-type function from the effects of its reduction rather than its total absence. The problem was well illustrated by the effects of homoeotic mutations on the nervous system of *Drosophila* (J. Palka, Washington University). Homoeotic mutations transform one entire structure into another—for example, wing into haltere; but the innervation of the

mutant structures seems to bear no consistent relationship to the target tissue, since it can correspond either to that of either the wild-type or the mutant appendage, or to a mixture of the two, sometimes in conjunction with an abnormal pattern unlike either.

Genetic mosaics, which can be used to circumvent the lethal consequences of developmental mutations, can also distinguish direct from indirect effects of such mutations. Myerowitz and Kankel (as reported by F. D. Ready (Stanford University Medical School)) have found, for example, that a small group of mutant retinula cells in the eye of *Drosophila* mosaics causes a 'cascade' of abnormal projections in the first and second optic ganglia, although all the other cells involved are wild type.

A similar 'cascade' seems to characterise the visual system of the Siamese cat, in which a discrete subset of optic nerve fibres which projects ipsilaterally in normal animals instead crosses at the chiasm. This gives rise to abnormal connections in the lateral geniculate and at the cortex, where the callosal connections have now been shown to be abnormal as well (C. Shatz *J. comp. Neurol.* **171**, 229; 1976). This abnormality is common to albinos of all species so far tested and is known to result from the absence of pigment rather than from the action of the albino gene itself, or the tyrosinase deficiency which is its direct effect. (The incrimination of the pigment is due to numberless mink-breeders, who have produced animals synthesising pigment in varying quantities through mutations in no less than five different genes; and to R. W. Guillery, who has established that the degree of optic fibre misrouting in minks corresponds to the pigment deficiency and not to any particular gene.)

Guillery (University of Wisconsin), following up this clue to the cause of the mis-specification of the retinal fibres, has found that (in hamsters) a small group of pigment epithelium cells is born and dies at the base of the optic stalk at a time when no other pigment tissue has yet appeared, but the optic fibres are just beginning to grow out from the eye. It seems possible that those pigment cells induce the specification of the ganglion cell axons.

Another example of induction of neural function by non-neural cells is that of the induction of biochemical differentiation in superior cervical ganglion cells *in vitro*. Rat sympathetic neurones cultured on their own are adrenergic, but when grown in medium 'conditioned' by various types of non-neural cell they synthesise acetylcholine and form nicotinic cholinergic synapses (Furshpan *et al. Proc. natn.*

Acad. Sci. U.S.A. **73**, 4225; 1976). Further experiments with these cells (P. Patterson, Harvard Medical School) have shown that all tissues seem to be able to induce cholinergic function, though to widely varying degrees which do not seem to be related to whether they normally receive cholinergic or adrenergic innervation, or any innervation at all. There is evidence in the spinal cord *in vivo* that transmitter induction is determined by position (Le Douarin *et al. Devl Biol.* **41**, 162; 1974).

The question of how far neural development is dictated by other tissues arises in a more specific form in the context of synaptogenesis, which has been investigated principally at the neuromuscular junction. Do neurones, for example, recognise their target tissue by its neurotransmitter receptors? Experiments showing synaptogenesis in the face of receptor blockade imply that if they do, it is not by virtue of their transmitter binding sites (Cohen *Brain Res.* **41**, 457; 1972; Anderson *et al. Neurosci. Abstr.* **2**, 707; 1976). The possibility, fleetingly raised, that receptor clusters (hotspots) might attract innervating fibres has also been ruled out by Anderson *et al.*, who showed by fluorescence labelling of receptors during synaptogenesis that innervating fibres did not make synapses at existing hotspots but induced receptor clustering very rapidly on contact.

It is now clear that an important contribution to the pattern of synapse formation is made by the withdrawal of most of the contacts initially made by the innervating neurone. The evidence for this selective elimination comes both from physiological and anatomical investigations on the neuromuscular junction (Brown *et al. J. Physiol., Lond.* **261**, 387; 1976) and from anatomical studies on the cat visual system (Rakic *Nature*, **261**, 467; 1976; Hubel *et al. Phil. Trans. R. Soc.* in the press). Its mechanism is not known, but seems to reside largely in the nerve. Brown *et al.* have ruled out competition between lenses by the demonstration of withdrawal of synapses from rat soleus after 75% of the innervating axons have been cut, even when that leaves many fibres denervated. They do not however rule out some influence of the muscle itself in the selection of which fibres are to be retracted.

Investigation of this phenomenon in nerve-nerve synapses has been made possible for the first time by the discovery of J. Lichtman, reported by D. Purves (Washington University), that four out of five terminals made by guinea pig submandibular ganglion cells are withdrawn within four weeks after

birth.

The broader implications of selective synaptic elimination are suggested by the proposal (D. Hubel, Harvard Medical School) that the period over which it occurs may correspond to the 'critical' period within which the development of the central nervous system can be influenced by its input. But the demonstration that the effects of monocular deprivation can be reversed during the critical period implies that fibres must be able to sprout as well as to withdraw.

So far, anatomical evidence of plasticity has been available only for the distribution of ocular dominance in the innervation of the visual cortex and anatomical data on the distribution of orientation selectivity (the nature of whose plasticity has been fiercely disputed) have been beyond neurology's technical means. Now however the anatomy of the orientation columns has become accessible through the discovery that ¹⁴C-deoxyglucose can be used for the selective staining of active neurones (Kennedy *et al. Proc. natn. Acad. Sci. U.S.A.* **73**, 4230; 1976). Hubel has applied this technique to the autoradiographic mapping of the vertical orientation columns of the monkey visual cortex, confirming the indications from electrophysiological mapping that there is no regular anatomical relationship between the orientation columns and the ocular dominance columns. Whether this technique can be brought to bear on the issue of plasticity in orientation selectivity remains to be seen.

In the meantime, however, invertebrate neurophysiologists are preparing to join the fray. R. Murphey (State University of New York) reported that unilateral immobilisation of the mechanoreceptive hairs on the cerci of developing crickets raises the sound threshold of the medial giant interneurone which receives its input from the cercal mechanoreceptors on the treated side of the animal. Matsumoto and Murphey (*J. Physiol., Lond.* in the press) suggest on the basis of the directional tuning curve of the giant neurone that an increase in the activity of the crossed inhibitory input from the contralateral cercal hairs may at least partly account for their result.

While this has interesting echoes of the suggestion (Duffy *et al. Nature* **260**, 256; 1976) that in monocular deprivation, a tonic inhibitory input to the visual cortex from the non-deprived eye may explain the unresponsiveness of deprived cortical cells, there is much too much uncertainty about the precise mechanism of either the invertebrate or the vertebrate results to justify firm conclusions, let alone direct comparison, at this stage. □

articles

Thermal evaporation of gas within galaxies by a hot intergalactic medium

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High-temperature ($T \sim 10^8$ K) intergalactic gas interacts with cooler embedded gas within galaxies and extragalactic clouds principally by thermal evaporation. From Cowie and McKee's model for evaporation of spherical interstellar clouds, in pressure balance with a hot ambient medium, we produce solutions for oblate and prolate spheroidal symmetry. The results are applied to evaporation of gases within spiral and elliptical galaxies, and can be extended to evaporation of filamentary clouds in the interstellar medium.

THE recent discovery of Fe XXV emission lines in the spectrum of X-ray emission from the Perseus cluster¹, together with measurements of the cosmic microwave background², thermal bremsstrahlung spectrum fitting³, and tailed radio galaxy measurements⁴, provides convincing evidence for the existence of a hot ($T \sim 10^8$ K) intergalactic gas within the potential well of rich clusters of galaxies. It is also likely that a substantial amount of gas, residual from the galaxy formation process, lies outside the rich clusters; such gas may provide the closure density required to bind the universe gravitationally. Field⁵ has summarised the observational constraints on such gas and shown that for a Hubble constant of $50 \text{ km s}^{-1} \text{ Mpc}^{-1}$, (a critical hydrogen number density of $2.6 \times 10^{-6} \text{ cm}^{-3}$), observations require only that the gas be highly ionised at $z = 2$ and at temperatures of less than 4×10^8 K at the present epoch. (For higher values of the Hubble constant, constraints imposed by the isotropic X-ray background are more severe, since the intensity is proportional to H_0^3). The most attractive evidence for the presence of such an intergalactic medium is the feature observed at 30 keV in the isotropic X-ray spectrum which may be fitted well by thermal bremsstrahlung from a gas with closure density and a present temperature of 3×10^8 K (refs 5, 6).

Several studies have been made of the thermal evaporation of gas within galaxies⁷⁻⁹, and of the ionisation by the metagalactic ultraviolet radiation which dominates at lower temperatures¹⁰. De Young⁷ and Bergeron and Gunn⁸, analysed the evaporation as direct particle heating by penetrating particles (ions and electrons) from the intergalactic medium, neglecting the dynamics of the evaporation process. An analytical model for evaporation has been developed by Cowie and McKee¹¹, who derived time-independent evaporative mass loss rates for a spherical cloud embedded in an infinite hot gas with asymptotic density n_i and temperature T_i by finding simultaneous eigenvalues of the mass and energy conservation equations

$$\nabla \cdot (\rho \mathbf{w}) = 0 \quad (1A)$$

$$\nabla \cdot (\rho \mathbf{w}^2 + 5/2 c^2 \rho) + \nabla \cdot \mathbf{q} = 0 \quad (1B)$$

where ρ is the mass density, $\mathbf{w}$ the velocity of outflow, c the isothermal sound speed and $\mathbf{q}$ the electron heat flux.

In the case in which the heat flux can be written as

$$\mathbf{q} = -\kappa \nabla T \quad (2A)$$

where κ is the classical thermal conductivity

$$\kappa = k_0 T^{5/2} = (1.84 \times 10^{-5} T^{5/2}) / (\ln \Lambda \text{ erg s}^{-1} \text{ deg}^{-1} \text{ cm}^{-1}) \quad (2B)$$

($\ln \Lambda$ is the Coulomb logarithm, ref. 10) the pressure is approximately constant throughout the interface and the flow subsonic; since the condition $M^2 \ll 1$ is satisfied, equations (1A and 1B) are approximately separable in a number of coordinate systems. (The equations cease to be separable when the term $(\mathbf{w} \cdot \nabla) \mathbf{w}$ in the momentum conservation equation becomes important.)

Here we shall describe the solutions for both oblate and prolate spheroidal symmetry. The analysis closely follows that in Cowie and McKee¹¹; in particular, oblate (prolate) spheroidal surfaces of constant u replace surfaces of radius r (the notation is that of Arfken)¹³. Thus the boundary conditions become $T = 0$ at the surface of the cloud ($u = u_0$) and $T \rightarrow T_i$ as $u \rightarrow \infty$. In terms of u_0 the mass loss rates from the clouds are

$$\text{Oblate: } \dot{M} = 8\pi a \mu k_0 T_i^{5/2} / 25 k_B (\pi/4 - \theta_0) \quad (3A)$$

$$\text{Prolate: } \dot{M} = 16\pi a \mu k_0 T_i^{5/2} / 25 k_B |\gamma_0| \quad (3B)$$

Here μ is the mean mass per particle, k_B the Boltzmann Constant, while

$$\theta_0 = \tan^{-1} (\tanh \frac{1}{2} u_0) \quad (4A)$$

$$\gamma_0 = \ln (\tanh \frac{1}{2} u_0), \quad (4B)$$

where a is related to the semi-major (l) and semi-minor (b) axes of the ellipsoid of constant u by

$$l = a \cosh u \quad (5A)$$

$$b = a \sinh u \quad (5B)$$

In the limit as the surface $u_0 \rightarrow 0$ (oblate spheroid $\rightarrow$ disk, prolate spheroid $\rightarrow$ needle), the result for the oblate case is a factor of $2/\pi$ times the mass loss rate from the surface of a sphere of radius a ; however, in the prolate case, the mass loss rate tends to zero as the surface area of the cloud vanishes. Both mass loss rates drive clouds towards sphericity on timescales comparable with the evaporation time.

We have also evaluated the temperature distributions as

$$\text{Oblate} \quad (T/T_f)^{5/2} = \frac{\theta_o - \theta}{\theta_o - \pi/4} \quad (6a)$$

$$\text{Prolate} \quad (T/T_f)^{5/2} = \frac{\gamma_o - \gamma}{\gamma_o} \quad (6b)$$

where θ and γ refer to the surface of constant u at which the temperature is evaluated. These may be used to evaluate the column density of a given ion in the evaporative interface (see Section III of McKee and Cowie¹⁴ for details). For example, along the axis $\nu = \pi/2$ of the oblate spheroid (the axis of rotational symmetry), we obtain for the column densities of OVI, assuming cosmic abundances¹⁵ and steady-state ionisation equilibrium¹⁶

$$N_{\text{OVI}} = 2.04 \times 10^{-4} a n_f (3 \times 10^5 \text{ K}/T_f)^{3/2} \quad (7)$$

Emission measures may also be calculated in terms of the external density and temperature (n_f , T_f) and the dimension a .

The mass loss rates calculated above may overestimate the mass loss if the heat flux given by the classical formula of equation (1) exceeds the maximum heat flux which may be carried by the electrons. This was estimated by Cowie and McKee to be $5\phi_s \rho c^3$, where ϕ_s is a factor of order unity. In the spherical case the mass loss rate may be calculated exactly even in this limit (compare Cowie and McKee¹¹); however, in the geometries considered, saturation of the conductivity is a symmetry breaking effect which precludes an analytic solution, while, as we have discussed above, the hydrodynamic equations are no longer separable.

Thus we note that, in the oblate spheroidal geometry, saturation of the conductivity occurs initially along the $\nu = 0$ axis, where temperature gradients are a maximum. In terms of the parameter

$$\sigma_o = \frac{2\kappa_f T_f}{25\phi_s \rho_f c_f^3 a} \simeq \left(\frac{T_f}{1.54 \times 10^7} \right)^2 a_{\text{pc}}^{-1} n_f^{-1} \quad (8)$$

which measures the ratio of the electron mean free path to the characteristic dimension of the system, this occurs when

$$\sigma_o \sim 5.7 u_o^{4/5}, u_o \ll 1 \quad (9)$$

or substantially earlier than would be the case for a sphere of radius a where saturation occurs at $\sigma_o \sim 1$. Initially the effect is confined to a very narrow range of ν and has little effect on the mass loss rates. As $\sigma_o \rightarrow 2.5$ the heat flux saturates throughout the interface; beyond this range we would expect the mass loss rates to approximate those found by Cowie and McKee¹¹ for spherical clouds of radius a , because the energy flow is governed by the outer regions of the saturated zone which is approximately spherically symmetric.

For clouds of a given column density, the effects of radiation on the evaporation depend only on the external temperature¹⁴; numerical integration shows that the results of McKee and Cowie for the radius of spherical clouds at which radiation dominates the conductive heat flux and clouds condense are approximately applicable to the oblate spheroidal geometry, if the dimension a is substituted for the spherical cloud radius. For temperatures $> 10^5$ K, the condition for radiation to be unimportant is that the factor σ_o exceeds 0.027. We have also assumed in the above discussion that the effects of magnetic field structure do not substantially inhibit the thermal evaporation. Cowie and McKee suggest that this approximation is reasonable if the magnetic fields of the cloud and intercloud are well connected, a condition that will be self consistently satisfied if evaporation is important. For the present purposes it may be considered as an extreme assumption, providing an upper bound to the mass loss rate by evaporation.

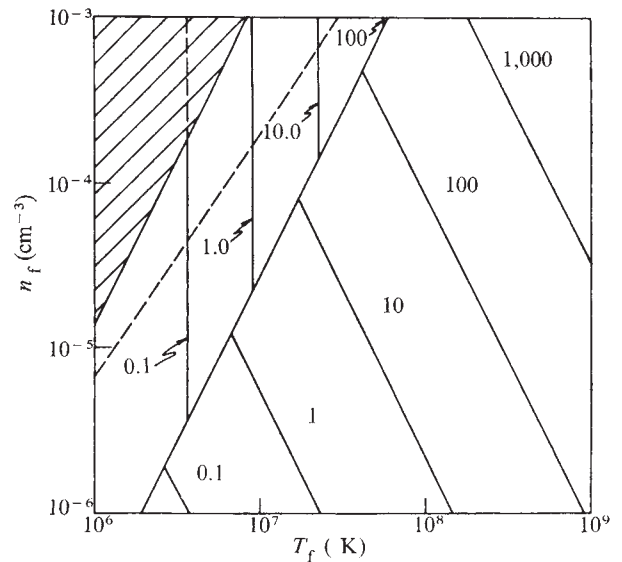


Fig. 1 Evaporative mass loss rates (in $M_\odot \text{ yr}^{-1}$) from a spiral galaxy of radius 15 kpc as a function of the local intergalactic temperature and density. The diagram is divided into three zones; in the shaded region radiation from the interface is important and galactic material condenses on to the galaxy, in the central region classical mass loss rates are applicable, while to the right of the solid line flux limiting effects are important (see text). We also show (— — — —) the value of the external parameters for which absorption lines in the interface may be detectable ($N_{\text{OVI}} = 10^{13} \text{ cm}^{-2}$).

We note finally that the evaporation rates given above for clouds in pressure equilibrium with the ambient gas should also be applicable to the evaporation of gas within galaxies, because, if the external temperature of the gas is large by comparison with the velocity dispersion of the galaxy, temperatures in the interface rise too rapidly for the gravitational field of the galaxy to be important.

In Fig. 1 we show the mass loss rate from a spiral galaxy of disk radius 15 kpc and thickness 200 pc for a wide range of intergalactic temperatures and densities. For $\sigma_o > 1$ (shown by the dashed line), we have used the spherically symmetrical results of Cowie and McKee; the discontinuity across this surface reflects the uncertainty in the mass loss rates. To within the factor of $2/\pi$ introduced by the geometry, the results may also be applied to galaxies of arbitrary ellipticity.

The mass loss rates are a sensitive function of the local temperature, but somewhat less sensitive to the local density. The existence of cool gas within galaxies therefore provides an upper bound to the local temperature of the intergalactic gas which is a weak function of the intergalactic density. The most sensitive probe is provided by the observations of column densities of $2-3 \times 10^{19} \text{ cm}^{-2}$ ($0.18-0.27 M_\odot \text{ pc}^{-2}$), of neutral hydrogen by Roberts and Whitehurst²² at 30 kpc from the centre of M31, where the surface density of material inferred from the rotation curve is $30 M_\odot \text{ pc}^{-2}$. The column density of ionised shielding slabs is unlikely to greatly exceed this value²⁴. In the absence of replenishment by the stellar population, the evaporation time for the neutral hydrogen alone equals the Hubble time at a local temperature of 3×10^6 K and is independent of the density for $n \geq 2 \times 10^{-6} \text{ cm}^{-3}$. This value is comparable to that obtained by Cowie and McKee⁹ from consideration of extragalactic clouds. The present results, however, are significantly more uncertain, as the possibility of substantial mass loss from a stellar population cannot be excluded. De Young⁷ estimates that if the $30 M_\odot \text{ pc}^{-2}$ is composed of $0.1 M_\odot$ M type dwarves losing mass at a rate of $10^{-12} M_\odot \text{ yr}^{-1}$ comparable with the solar value, an upper bound to the mass loss rate is $3 \times 10^{-10} M_\odot \text{ yr}^{-1} \text{ pc}^{-2}$. This would result in the weaker condition $T \leq 10^7$ K for $n = 3 \times 10^{-6} \text{ cm}^{-3}$.

It must be stressed, however, that these values refer only to the temperature of intergalactic gas within local groups. We emphasise that the average temperature of the intergalactic gas may be substantially higher, though temperatures as high as 3×10^8 K, required to explain the isotropic X-ray background, seem to be ruled out by the upper bounds on cooler material within groups, provided by soft X-ray measurements (compare Cowie and McKee⁹). The values of the mass loss rates from the galaxies are sufficiently large that evaporative stripping could clear material from galaxies in regions where intergalactic gas temperatures are high, and could account for the absence of gas within field SO galaxies.

We note that for local intergalactic densities of order 3×10^{-6} cm⁻³ neither emission nor absorption in the interface will be detectable, irrespective of the ambient temperature. If lower temperature, higher density gas does exist within the local cluster, the interface might be detectable in absorption (Fig. 1).

We may apply similar arguments to galaxies which are members of rich clusters. The parameters of the intracluster medium in the central regions of the cluster, based on the thermal bremsstrahlung interpretation of the X-ray emission from the Coma cluster¹⁸ are $n \sim 3 \times 10^{-3}$ cm⁻³ ($\Omega \sim 1,000$), and $T \sim 10^8$ K. The ratio of the mass loss rate by thermal evaporation to the stellar mass injection is maximised if the cool gas is confined to the nucleus of the galaxy. The evaporation from the nucleus however, is $1.4 \times 10^{-4} R_p^{1.625} M_\odot \text{ yr}^{-1}$ (Fig. 1), where R_p is the core radius of the galaxy in pc; for $R_p = 100$ the mass loss rate is $0.25 M_\odot \text{ yr}^{-1}$. Thus even in the most favourable case for retaining gas, the evaporation rate exceeds the probable mass injection rate by stars inside the nucleus for all but the most massive galaxies.

We suggest that this effect rather than ram pressure stripping is responsible for the absence of the OII $\lambda 3727$ line in elliptical galaxies within rich clusters, because, as Gisler²³ has pointed out, ram pressure stripping is ineffective in removing pre-existing gas from the nuclei of galaxies. In a sample of 100 galaxies within the Coma cluster by Sargent and Gunn (quoted

in ref. 19) this line was detected only in the giant central galaxies NGC4889 and NGC4874, which is consistent with the present results.

Finally, we may note that comparison of the critical radius for radiative stabilisation with characteristic scales of the galaxies (Fig. 1) indicates that radiation is never important, even for the giant central galaxies. The results indicate that the cD galaxies are not now growing by conductive condensation of hot intracluster gas, as has been suggested¹⁹, although radiative cooling of the central intracluster gas on scale lengths large compared with the radii of these galaxies may ultimately result in gravitational accretion of material on to these galaxies^{21,25}.

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Geochemical evolution during fractional crystallisation of a periodically refilled magma chamber

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A realistic model of volcanic plumbing predicts that most of the established major, trace and isotopic chemical features of the common basalts could have been imposed during magma evolution in high-level magma chambers, for which process there is extensive independent field and phase equilibria evidence. These effects must be quantified before chemical features of lavas are ascribed to the nature of the underlying upper mantle.

THE changes in the chemistry of basaltic liquids which occur during a variety of partial melting processes have been explored in mathematical models^{1,2}. Extensive variations in the concentrations of those incompatible trace elements which are strongly concentrated into the liquid are observed to occur with little accompanying change of the concentrations of the major elements (notably Fe, Mg, Na, Ca), particularly where the percentage of partial melt is small. Similar results can be

produced by interaction of basalt magma with a peridotite wall rock in a process akin to zone refining³. General geological constraints suggest that both partial melting and zone refining processes have been restricted to relatively high pressure environments in the Earth.

The argument outlined here is developed in the particular context of basaltic magmas but the model presented is equally applicable to any magma, because it is a mathematical formulation of the effects which will follow from a particular style of plumbing in a volcano.

Basaltic liquids which are isolated from their upper mantle source regions may undergo crystallisation either while full equilibrium is maintained between the residual liquid and the crystals which have formed, or in such a way that all crystals formed are immediately removed from contact with the liquid. The changes in the chemistry of the liquids during these processes differ strikingly from those produced in partial melting processes. Large variations in the concentrations of the incompatible trace elements can occur, but only at the expense

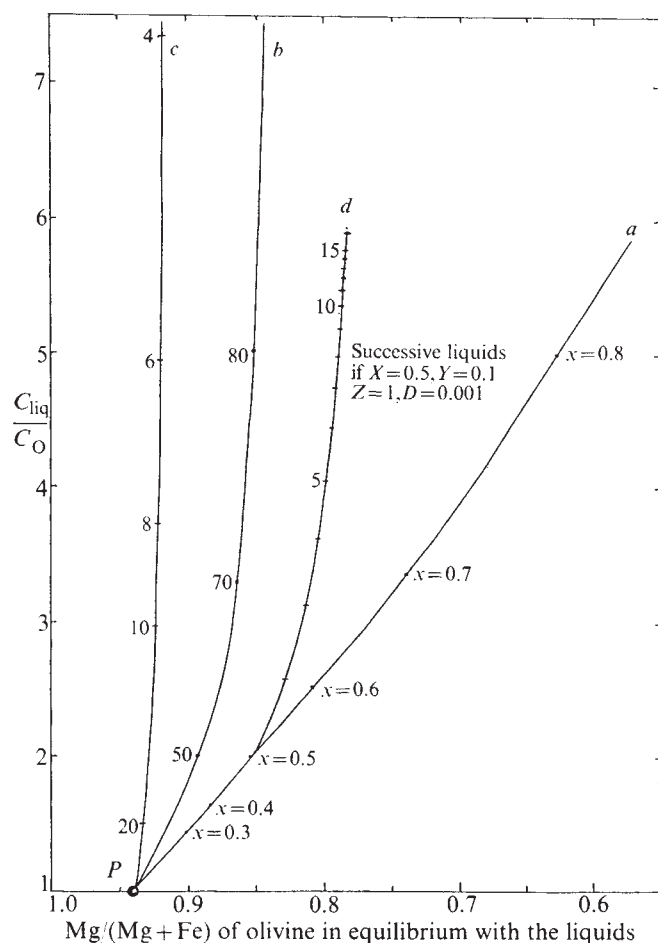


Fig. 1 How the relative concentration of an incompatible trace element: ($D = \frac{\text{conc. element in crystals}}{\text{conc. element in liquid}} = 0.001$) will vary in the liquid fraction during a variety of processes which may lead to the development of a liquid *P*, and a further group of processes which may act on a liquid such as *P*. The change of concentration of the incompatible trace element relative to its concentration in liquid *P* is plotted as a function of the ratio of two major, relatively compatible elements, exemplified by Fe, Mg for which it has been assumed that $D = 0.7, 2.1$, respectively. The scale is, however, translated into one showing the composition of the olivine which would coexist with these liquids, assuming

$$K_D = 0.33 \dots \left(\frac{\text{Fe}}{\text{Mg}} \right)_{\text{ol}} \left(\frac{\text{Mg}}{\text{Fe}} \right)_{\text{liq}}$$

It has been assumed that liquid *P* is in equilibrium with olivine of composition Fo_{84} . Further discussion is in the text.

of producing large volumes of crystal cumulates, and extensive increases in the ratios of major elements such as Fe/Mg, Na/Ca. General geological constraints suggest that such processes occur extensively at low pressures, and are probably important at high pressures also.

The differences between the chemistry of the liquid products of the partial melting, equilibrium and fractional crystallisation processes are illustrated in Fig. 1, curves *a*, *b*, *c*. Curve *a* shows the evolution of the residual liquid composition during closed system perfect fractional crystallisation (with percentage crystallisation marked). Curve *b* shows the evolution of the intercrystalline liquid during perfect equilibrium crystallisation (with percentage crystallisation marked). Curve *c* shows the variation during an equilibrium partial melting process which would yield *P* as a 30% melt of the source region (with intermediate compositions and the percentages of melt then formed indicated).

None of the processes so far considered are effective in causing a variation in the ratio of two elements which are both strongly concentrated into the liquid relative to the crystals.

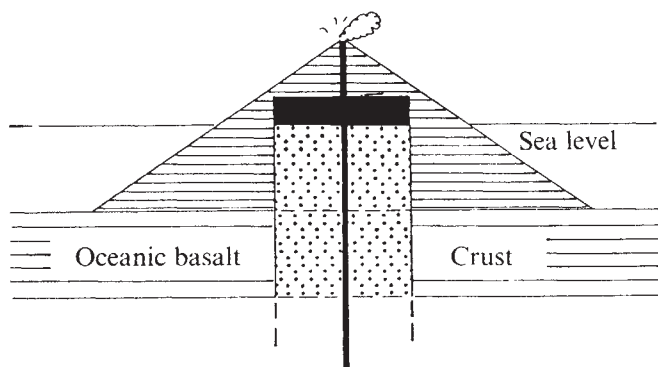
These considerations have led to the development of two criteria of widespread application in studies of basalt genesis and upper mantle motions. If the concentrations of incompatible elements vary greatly in a series of basalts of comparable major element composition, this variation reflects differences in the extent of partial melting at the source region which gave rise to each magma batch. If the ratio of the concentrations of two incompatible trace elements differs between two series of basalts, they have been derived from two different source regions of distinctive chemistry (partly characterised by the ratios of the incompatible trace elements).

These criteria have been critical in underpinning the hypothesis of the existence of hot fertile plumes of upper mantle material rising beneath volcanic 'hot spots'. Their application carries with it the implicit assumption that there has been little fractional crystallisation of the basalts near the surface, and that little chemical change, particularly of the trace element ratios, can have occurred in those near-surface processes. This view apparently conflicts with a wealth of observational and experimental data which indicate that most erupted basalts have been thoroughly modified in composition during near-surface processes⁵⁻⁹.

The evolution of basaltic magmas by equilibrium or fractional crystallisation in closed systems provides only one model of what could occur in near-surface magma chambers. A slightly more sophisticated model has recently been explored in which the magma in a high-level chamber undergoes continuous fractional crystallisation, but is periodically fed with a new batch of the parental magma from depth. In this model, the arrival of this new magma batch is assumed to displace a sample of the residual liquid from the chamber as a lava flow. The rest of the residual liquid and the new batch of parental magma then mix, and the fractionation process continues.

Many central volcanoes seem to have a core of gabbroic cumulates extending to great depth¹⁰⁻¹⁷. In the case of oceanic island volcanoes it is manifest that much of what is now plutonic gabbro must have replaced what was previously basalt which had erupted into and had the opportunity to react with seawater. A thick portion of the oceanic crust formed at the ridges is composed of gabbro which may largely have replaced previously erupted basalts. As a refinement to the simplest model, provision has been made to consider the possible effects which are due to the digestion of previously erupted basalts

Fig. 2 Model of the evolution of an oceanic island volcano. The active magma column and differentiating chamber are shown in black. The advancing magma chamber has digested all the previously erupted basalts in the stippled column, replacing them with cumulates. Incorporation of basalts altered by contact with seawater has contaminated the isotopic ratios of later erupted basalts. Because the cumulate pile is built up below the active and advancing magma chamber, this may be a relatively thin sheet of liquid at the cumulate-roof interface. There need never have been a magma chamber of the size of the ultimate gabbro pile.



(Fig. 2). This adds a process akin to zone refining on to the other effects. (This zone refining, however, operates within the fertile ground of previously erupted basalt rather than in the less promising ground of upper mantle peridotites).

Let X = mass fraction of cumulate formed from the magma in each cycle

Let Y = mass fraction of the original liquid before this fractionation which is extracted as a lava flow at the end of each cycle

Let Z = mass of parental magma added in each cycle (expressed as an absolute quantity relative to the initial size of the magma chamber)

Let C_0 = the initial concentration of an element in the parental liquid

Let C_B^S = concentration of element in the residual liquid of the fractionation cycle, in the steady state.

Let D = $\frac{\text{concentration of element in crystal extract}}{\text{concentration of element in liquid}}$

Let C_Q = concentration of element in the material digested by the magma chamber

Let q = fraction of the total cumulate X fraction, which has been precipitated in order to provide the energy necessary to digest a mass fraction Q of previously erupted basalt. It has been assumed that $Q = qX$ and that $(1-q)X$ represents fractionation caused by conductive and other energy losses.

The following relationship may then be derived. (Full details of these derivations will be given in a longer communication which is in preparation.)

The steady-state mass, M , of the magma chamber is given by $Z/(X - qX + Y)$, M and Z being expressed in any absolute or relative units.

The way in which the potential lava composition, C_B , evolves in successive cycles towards the steady-state composition, C_B^S , during successive cycles of the addition, mixing and fractionation process, is shown for just one choice of the parameters X , Y , in Fig. 1, curve d ($q = 0$). As X becomes smaller, curve d approximates more closely to c . As X/Y becomes larger, the steady-state composition (C_B^S) moves to higher values of C^S/C_0 .

The concentration of an element in the basalt erupted periodically when the magma has reached a steady state, relative to its concentration in the parental magma, is then given by

$$\left(\frac{C_B^S}{C_0}\right) = \frac{\{X[1+q((C_0/C_0)-1)]+Y\}(1-X)^{p-1}}{1-(1-X-Y)(1-X)^{p-1}} \quad (1)$$

(provided Z , C_0 remain constant and assuming Rayleigh fractionation).

Figure 3 plots (C_B^S/C_0) against D for a various values of X , Y , assuming always that $q = 0$. The dotted curves a, a' show the values of D for which one incompatible element will achieve a steady-state concentration 1.5 times greater than that achieved by an element for which $D = 0.1, 0.2$ (lines b, b') for cases where $Y = 0.001$. Much larger (absolute) differences in D are necessary to produce the same effect when D lies closer to unity and separation of elements of similar D value is most effective when $X \gg Y$. When X is large relative to Y , and X, Y, D are all small, it is possible to produce large variations in the ratio of two incompatible elements without large change in the major element composition of the parental magma. The values of X, Y are critical in determining the steady-state concentrations of elements with D very different from unity.

Note that compatible element concentrations ($D \sim 0.4-2.5$) are relatively insensitive to the absolute and relative values of X, Y , especially when X, Y are small and $X > Y$ (Fig. 3).

The X/Y ratio determines the relative concentration achieved by highly incompatible elements, but the absolute values of X and Y become very important as D increases. The larger the values of X, Y , the higher C_B^S/C_0 of elements for which $D \sim 0.3$, and the lower C_B^S/C_0 of elements for which $D > 1$. In other words, the absolute values of X, Y have little effect on C_B^S/C_0

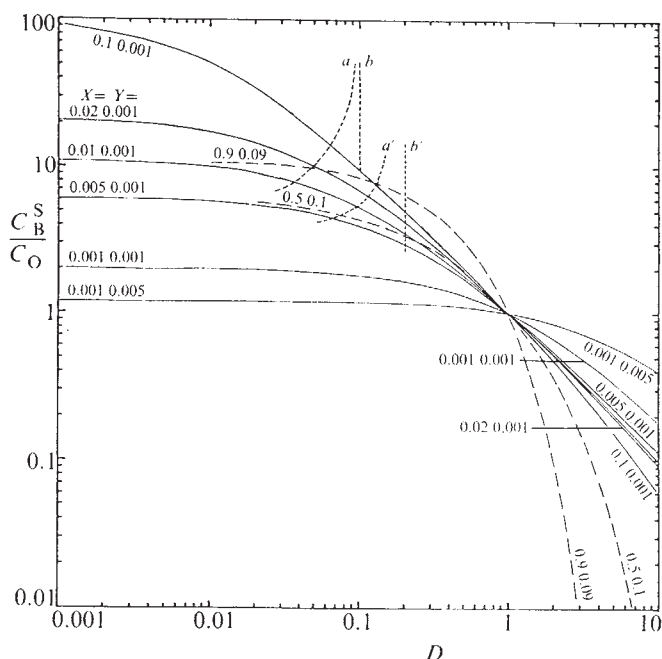


Fig. 3 Variation of the relative concentration of an element (C_B^S/C_0) as a function of D for a selection of choices of X, Y , ($q = 0$ in equation (1)). At values of D along curves a, a' , the concentration of an incompatible element is 1.5 times that at values of D given on b, b' , for all curves with $Y = 0.001$.

$$\frac{C_B^S}{C_0} = \frac{(X+Y)(1-X)^{p-1}}{1-(1-X-Y)(1-X)^{p-1}}$$

Further discussion is in the text.

for incompatible elements, provided X/Y is constant, but greatly influence the concentration of a highly compatible element such as nickel or chromium, the steady-state concentration remaining higher as the absolute values of X, Y decrease.

The major geochemical effects hitherto considered to be indicative of small and variable degrees of partial melting or derivation from different upper mantle source regions can also be produced during plausible high-level fractional crystallisation processes for whose operation there is so much independent evidence. This type of fractionation could be effective in exploiting small differences in D (as between isotopes of a single element) to produce small but significant differences in their relative concentrations. (These differences will not be completely removed by the normalisation procedures in standard use unless the differences in D for different isotopes vary linearly with mass.)

The concentration, C_B^S of an element in the steady-state liquid is made up of a spectrum of partial concentrations, K_p , each arising from one previous input of parental magma, such that

$$\sum_{p=1}^{p=P} \frac{K_p}{C_B^S} = \sum_{p=1}^{p=P} [(1-(1-X-Y)(1-X)^{p-1})] \times [(1-X-Y)(1-X)^{p-1}]^{p-1} \quad (2)$$

The number of mixing and fractionation cycles, p , for which this summation is equal to 0.5 represents the average residence time (in cycles) of that particular element in the magma chamber. The smaller the values of D, X and Y , the larger p becomes. An incompatible radioactive parent element (for example, Rb) may have been effectively present in the magma for a much longer time than its more compatible decay product (for example, ^{87}Sr).

Returning to equation (1), we can consider the probable effects of the digestion of previously erupted basalts. Setting $q = 0.5$, $C_0/C_0 = 2$, $X/Y = 1$, C_B^s will be 1.25 times as large as it would have been had there been no digestion of basalt. If $q = 0.8$, however, $(C_0/C_0) = 5$, $X/Y = 3$, C_B^s becomes 3.4 times larger than it would otherwise have been, and the contribution of digestion of previously erupted basalts begins to dominate the geochemical effects.

The potential effect of the digestion of previously erupted basalts, whose isotopic ratios have been altered by interaction with seawater, to influence the isotopic ratios in the later basalts to be erupted may be seen by rewriting (1) to obtain the fractional change in the isotopic ratio, Δ , where

$$\Delta = \left\{ \left(\frac{{}_1C_B^s}{{}_1C_B^s} \right) \left(\frac{{}_2C_B^s}{{}_2C_B^s} \right) - 1 \right\}$$

and ${}_1C_B^s$ = steady-state concentration of isotope 1 if $q = 0$, ${}_1C_B^s$ as here = steady-state concentration of isotope 1 if $q \neq 0$, and so on. Assuming no difference in D for the isotopes 1, 2, and setting the ratios of the two isotopes in the altered basalt

$$\frac{{}_1C_Q}{{}_2C_Q} = r \left(\frac{{}_1C_0}{{}_2C_0} \right)$$

then

$$\Delta = \left(\frac{X}{Y} \right) q \left(\frac{{}_2C_Q}{{}_2C_0} \right) (r-1) / \left\{ \left(\frac{X}{Y} \right) \left[1 + q \left(\frac{{}_2C_Q}{{}_2C_0} - 1 \right) \right] + 1 \right\} \quad (3)$$

Δ has been calculated for the values of q , C_0/C_0 and X/Y discussed above. If $r = 1.0014$ (the fractional change in $^{87}\text{Sr}/^{86}\text{Sr}$ for average ocean floor basalts deduced by Spooner¹⁸), then $\Delta \sim 5$ to 13×10^{-4} , which is of the order of size observed between fresh ocean floor basalt and ocean floor basalt and oceanic island tholeiites¹⁹.

Isotopic fractionation (with D values varying non-linearly with mass), larger effective residence times in the magma chamber for an incompatible parent element than for its radiogenic products, and digestion of previously erupted, isotopically altered, basalts could all contribute to changing the isotopic ratios of the basalts erupted at a later stage in the development of oceanic islands. The last of these processes apparently has the greatest potential. It could be that a substantial part of the differences in the isotopic ratios of Sr, Nd and Pb between basalts from hot spots relative to those from mid-ocean ridges may reflect the longevity and comparative geochemical fertility of seawater rather than of any underlying mantle source region.

Contamination by assimilation of crustal material could also occur during the creation of cumulate piles in central volcanoes of continental regions.

In discussing the possible effects of contamination on major or trace elements, or isotopes of elements, several relevant factors must be borne in mind. It is the local average continental crust, or isotopically altered oceanic crust (not seawater itself) which is causing the contamination and which must, therefore, be characterised. The advancing magma chamber will thermally metamorphose the assimilated materials before they are digested; there may be further isotopic exchange with expelled waters at this stage. The assimilation of basalt by basalt magma would create no dramatic change of major element chemistry of the magmas or the cumulates, but the assimilation of crustal material might well do so. The effects of this chemical contamination cannot be simply envisaged, however, because the speeds with which the added elements are removed to the cumulus pile and their steady-state concentrations in the magma are functions of the distribution coefficient for each element. Where the contamination is severe, the bulk distribution coefficients could themselves be modified due to the change in the nature or proportions of the minerals precipitating.

Thus far the effects of this model of magmatic evolution on trace elements, for which $D \sim \text{constant}$ have been treated. For the major components, D is not constant, changing progressively with crystallisation of a phase within any one equilibrium (for example, olivine + liquid) and changing abruptly

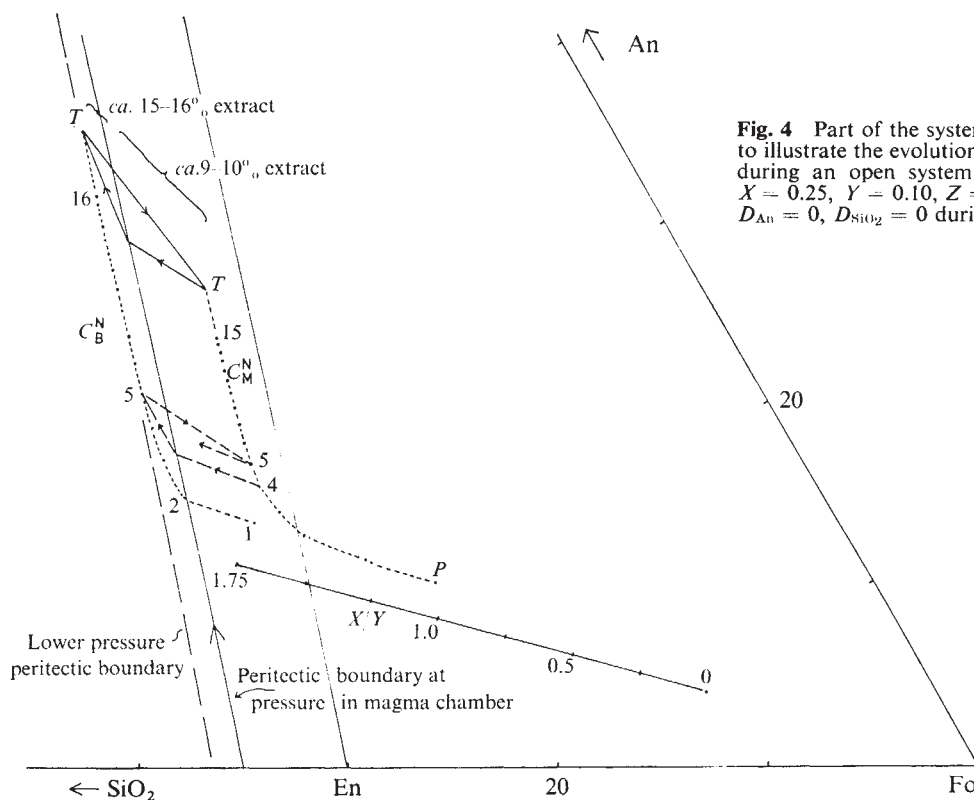


Fig. 4 Part of the system forsterite-silica-anorthite (simplified) to illustrate the evolution of the major components of the liquid during an open system fractionation process $C_0^N = C_0 = P$, $X = 0.25$, $Y = 0.10$, $Z = 1.0$, $D_{Fo} = \text{variable}$, $D_{En} = \text{variable}$, $D_{An} = 0$, $D_{SiO_2} = 0$ during Fo crystallisation only (discussed in the text).

whenever there is a change of equilibrium (for example, to olivine + pyroxene + liquid). The possible effects here are difficult to convert to mathematical models, but can be handled by graphical means. Figure 4 shows where the steady-state liquid composition will lie for two particular choices of parent liquid composition, and how one of them evolves during successive mixing and fractionation cycles to reach that composition.

With parental magma composition O , (Fig. 4), the steady-state liquid composition may lie anywhere along the line from forsterite through O , depending upon the ratio of X , the amount of crystals extracted, to Y , the amount of magma erupted.

With parental magma P the amount of fractionation supposed is sufficient for the residual liquids in successive cycles to evolve beyond the cotectic between pyroxene and forsterite. The evolution of the liquid towards the steady-state concentration has been traced graphically for one choice of X , Y . The actual evolution of the liquid between the fourth mixed magma (C_M), the fifth residual magma C_B , and the fifth new mixed magma (C_M) is illustrated by dashed lines. The loci (but not the path) of the evolution of liquids C_B , C_M to their steady-state compositions, T , are shown by dotted lines. The steady-state recycling by repeated fractionation and mixing is shown by a triangle of solid lines. The loci C_B , C_M approach each other as X becomes smaller. The steady-state compositions move further from O , P as X increases relative to Y .

The important general principles to be derived from Fig. 4 are that the steady-state liquid composition could be arrested within the liquidus field of a single crystalline phase (for example, olivine or pyroxene), or on a cotectic where two or more phases are precipitating together. In certain circumstances, such as those depicted in Fig. 4, which are relevant to the crystallisation of olivine from natural basalts at low pressures, steady-state residual liquid compositions are constrained to lie close to those of liquids which are in reaction relationship with olivine. This result contradicts the general rule in closed system fractional crystallisation, that residual liquids will depart in composition relatively rapidly from a peritectic relationship (see Fig. 4). To add to the possibly misleading aspects of the effect shown in Fig. 4, any drop in pressure which accompanies the eruption of a liquid from the magma chamber to the surface will expand the limits of the olivine liquidus field (Fig. 4), tending to place the locus of the peritectic liquids at low pressure even closer to the liquid compositions generated at a slightly higher pressure.

Summarising, in this open-system fractional crystallisation process, a near-surface magma chamber is fed regularly with batches of parental magma which mingle with the liquid already there. The mixed magmas undergo continuous fractional crystallisation. The steady-state lavas which may be extracted from time to time will then show the following characteristics: (1) Uniformity of composition, provided the thermal insulation of the magma chamber, the rate of supply, the amount and nature of assimilation and the composition of the parental magma are maintained constant. (2) Large differences of composition between the lava and the parental magma with respect to major and trace element concentrations and ratios. (3) Strong control of liquid composition by the low pressure phase equilibria. The steady-state liquid composition may be arrested within the liquidus field of one crystalline species only (specifically, that which would be the liquidus phase in the parental magma). It is, however, more likely to be located on a cotectic where two or more crystalline species precipitate together, or very close to a peritectic where one (or more) crystalline species is in reaction relationship with the liquid while others precipitate.

Steady-state lavas developed from the same parental magma but in magma chambers with different characteristics of heat loss and supply rate may show the further characteristics:

(4) Large variations in the concentrations of incompatible elements (for example, the large-ion, lithophile elements) with

little change in the concentrations of the more compatible major elements, and little variation in the iron: magnesium ratio.

(5) Significant variations in the ratio of two incompatible elements.

If the magma chamber advances ahead of the growing column of cumulates within the growing volcano in part by digestion of previously erupted basalts, then:

(6) Enrichment factors mentioned in (4) may be greatly enhanced in the later products of the volcano.

(7) Isotopic characteristics of the previously erupted basalts, which may have been altered by contact with sea water, will influence the isotopic characteristics of the later products of the volcano. Higher ratios of the radiogenic to non-radiogenic isotopes of Sr, Pb, Nd in the lavas erupted from large central oceanic island volcanoes than in ocean floor basalts may partly reflect the chemical characteristics of sea water. This process can explain the difference in strontium isotope ratios observed⁷ between oceanic island and oceanic floor basalts quantitatively as shown above. Data are lacking at present for isotopic ratios of Pb, Nd in the average altered oceanic basalt section, hence this part of the prediction cannot yet be tested.

Provided X , Y are small,

(8) Highly compatible elements (for example, Ni, Cr) may show relatively high concentrations despite the effects noted in (2), (4).

(9) The mass of the magma chamber is large relative to the periodic batches of parental magma added to it, and the composition of the steady-state magma will be insensitive to short-term random fluctuations in the mass or composition of the added batches.

Lastly,

(10) There is no requirement for the magma chamber to be large. The whole ocean floor gabbro sequence might be laid down from a magma sheet never more than a few tens of metres thick, as might many cumulus piles in central volcanoes.

As a general comment one may note that few of these features are anticipated as a result of closed system fractional crystallisation processes.

The geochemical features noted under (1), the second sentence of (3), (4), (5), (6), (7) or (8) can therefore only be used in support of claims that particular basalts are primary, have been derived by different degrees of partial melting of some source region, or have been derived from fundamentally different source regions, if the model of volcanic plumbing discussed in this paper can be specifically excluded. Because of the feature mentioned in (2), overlooking the effects of high-level fractionation which are possible results of this model will result in misleading conclusions about the chemistry of the source regions and the processes which have affected them. The hallmark which indicates that lavas may have evolved in this type of process is set out in (3)—often the lavas will be porphyritic, but in general will be close to saturation at low pressure with two or more phenocryst species.

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An oxygen-isotope climatic record from the Devon Island ice cap, arctic Canada

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Isotope measurements on two adjacent cores through the Devon Island ice cap provide a well-dated climatic record for the past 5000 yr. Fluctuations in annual values include much 'noise', and ice flow over a rough bed produces distortions in the lowest 5% of core which covers roughly 120,000 yr. Comparison with the Camp Century, Greenland, record helps to separate climatic changes from changes in ice thickness or flow pattern.

SINCE the $^{18}\text{O}/^{16}\text{O}$ ratio in polar snow depends on its temperature of formation¹, deep cores from polar ice sheets contain a record of past temperatures. Records covering all or part of the Wisconsin Glaciation have been obtained from Greenland and Antarctica²⁻⁵. We report here on two cores from the Devon Island ice cap, arctic Canada. These cover the same period as the Camp Century, Greenland, core, namely about 125,000 yr, but because the ice more than 10,000 yr old is compressed into a few metres near the glacier bed, the record is less detailed.

The $^{18}\text{O}/^{16}\text{O}$ ratio is expressed as the fractional difference between the ratio in the sample to the ratio in 'standard mean ocean water'. It is denoted by δ and measured in ‰. In polar snow δ is negative; the more negative, the lower the tem-

perature. Correlations between δ and temperature must be made with caution, however, because δ will also change if the source area of precipitation changes, if the ice thickness changes with time or if the ice at depth originates upslope from the drill site². Drilling near an ice divide, as in the present study, eliminates the last problem.

The ice cap and boreholes

Figure 1 shows the ice cap and borehole site some 600 km from Camp Century on the opposite side of Baffin Bay. The boreholes, Holes 72 and 73, are 27 m apart on the same flow line about 900 m north of the ice divide. Hole 73 is upslope from Hole 72. The holes reached bedrock at depths of 298.9 m (Hole 72) and 299.4 m (Hole 73). The ice-bedrock contact was sharp; the lowest metre of each core contained a few small dirt particles, probably of wind-blown origin, but there was no basal moraine in the ice. The temperature at the interface was -18.4°C . Annual precipitation is 0.22 m water equivalent and the mean annual air temperature is about -23°C . In most summers, a little surface snow melts and refreezes in the snow-pack. This, combined with the relatively low precipitation rate, means that seasonal variations in δ are eliminated in a few years.

Dating the ice

For a palaeoclimatic record to be of any value, the age of the ice at each depth must be known. Age t at depth y is calculated by the formula

$$t = \int_0^y v^{-1} dy$$

where $v(y)$ is the component of ice velocity perpendicular to the surface. In previous studies, various functional relationships between v and y have been assumed^{6,7}. In the present case, v was measured at various depths in the borehole⁸ and the integral evaluated numerically. Although a measured relation should be better than a theoretical one, the measured values must be assumed typical of past values. This implies that the ice cap has been in a steady state (no change in precipitation rate, ice thickness or temperature) for the time period covered by the core. This may be a reasonable approximation for a period of a few thousand years but not for one extending into the last glaciation. At any depth in a steady-state ice cap, the value of v , expressed as the distance an ice particle moves downward in 1 yr; should be equal to the thickness of an annual ice layer, a quantity that can be determined from seasonal variations in concentration of microparticles in the core⁹. Thirteen such measurements (10-yr means) in the youngest 2,100 yr of core checked satisfactorily.

The time scale was further checked by absolute dating of the ice at four points by the ^{32}Si method¹⁰ and at another four points by ^{14}C obtained by down-borehole extraction of CO_2 from air bubbles in the ice¹¹.

Fig. 1 Map showing location of boreholes on the Devon Island ice cap and its position relative to Camp Century, Greenland.

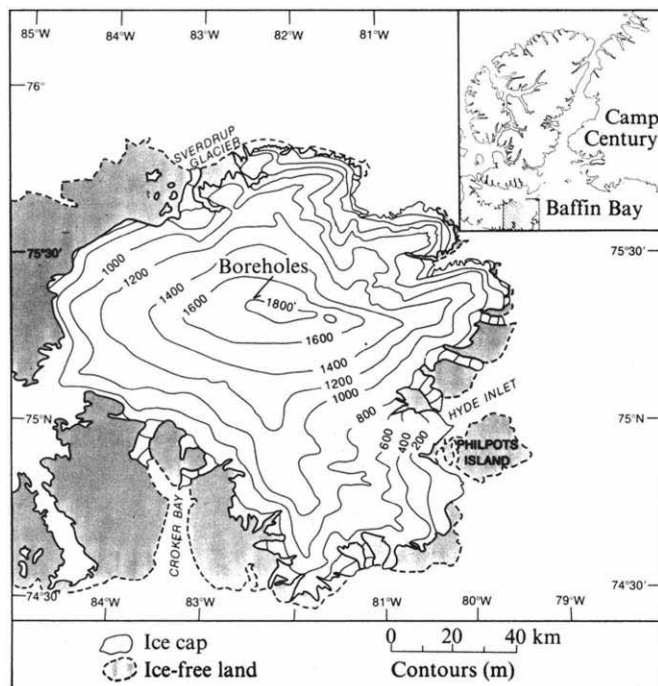


Figure 2 shows the time scale and absolute dates. The curve is within 1 s.e. of each of the ^{32}Si dates. The discrepancies that exist may be real rather than statistical because similar differences at about the same ages have been observed in two Greenland cores. This suggests that the production rate of ^{32}Si in the atmosphere may vary with time. The curve is also within 1 s.e. of two of the four ^{14}C dates and within 2 s.e. of another. The discrepancy of the youngest date is very large, however. We conclude that this ^{14}C date is wrong, probably because the sample was contaminated by fossil CO_2 . A $^{12}\text{C}/^{13}\text{C}$ analysis supports this idea. The standard error of the calculated ages, based on the standard error of measurement of ν , is about 6% for the first 1,500 yr and increases to about 20% at 5,000 yr. The precision of the ages is probably better than this because the curve agrees with most of the absolute dates. We therefore believe that the record for the past 5,000 yr is well dated.

Comparison of adjacent cores

Having two boreholes 27 m apart enabled the variation in δ over short distances to be measured. To check the repeatability of the mass spectrometer measurements, 400 samples from one core were measured twice. The correlation coefficient between measurements was 0.991; thus instrumental inaccuracies are negligible. Next, 450 samples, each of the calculated annual layer thickness, were cut from each core. The δ values were compared in equivalent segments corresponding to 50 calculated annual layers. The correlation varied between 0.45 and 0.72. These relatively low values show that much of the variation in annual values of δ is 'noise'. Part of it probably results from variations in snow accumulation over short distances so that samples cut to the mean annual layer thickness will contain varying amounts of summer and winter snow; the remainder probably results from variations in the amount of meltwater percolation.

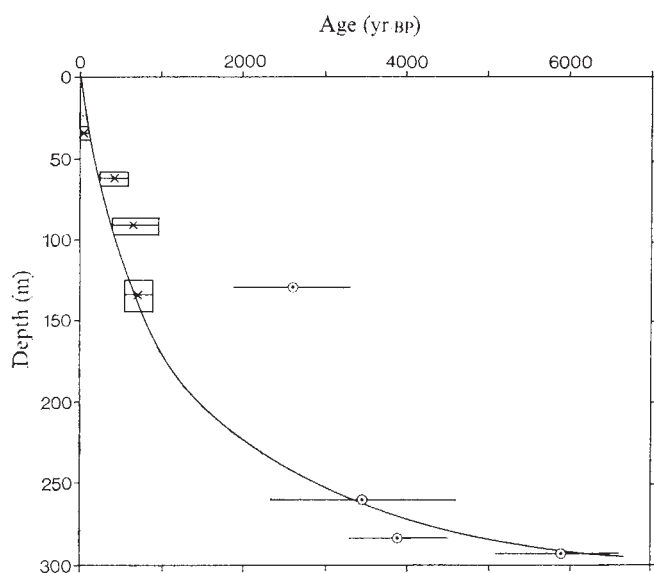


Fig. 2 Time scale for the core, based on measured vertical velocity, and absolute dates. The vertical lines beside the ^{32}Si dates indicate the depth interval over which each sample was taken. Each ^{14}C sample covers a depth of 1.5 m. X, ^{32}Si date; $\odot$, ^{14}C date. Bars are 1 s.e. on each side of mean.

The isotope profiles from the two boreholes closely resemble each other except near the base of the ice. The correlation between 50-yr mean δ values is 0.965 between the surface and 13 m above the bed, but only 0.449 between 13 and 5 m. The correlation decreases because bumps and hollows in the bedrock disturb the regular flow pattern in the ice immediately above.

Figure 3 shows the lowest 5 m of each record. Although the profiles show many of the same features, gaps in each are apparent. No gap is more than 50 mm long and construction of a composite record, described later, suggests that 25–30% of each record is missing. This assumes that no part of the record is missing from both cores, which may not be true. The extremely steep δ gradients at certain points may be additional evidence for gaps. For example, the feature at 2.6 m in both cores corresponds to a change of 9‰ in about 30 mm of core and the feature at 2.3 m in core 72 to a change of 5‰ in 10 mm. Because

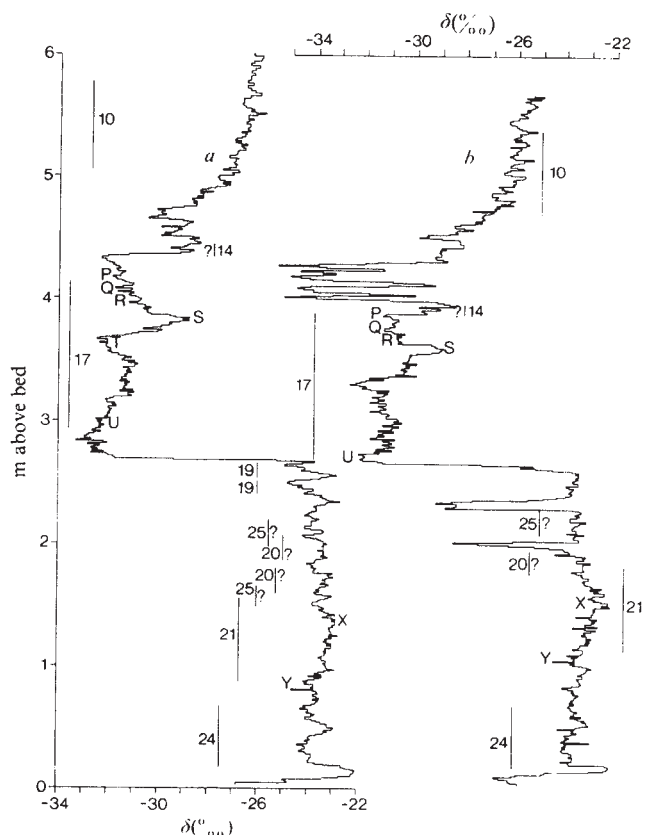


Fig. 3 Values of δ , for samples 10 mm long (a, core 73; b, core 72), against distance above bedrock for lowest few m of each core. Sections and features marked with the same number or letter are considered to be equivalent. The 'cold peak' at 2 m in Core 72 is doubtful; it may result from a sampling error. Gaps up to 50 mm long are apparent in both cores.

molecular diffusion reduces δ gradients, these values must have been greater in the past. We consider it most unlikely that such gradients represent climatic effects; a recent tectonic origin seems more probable. We emphasise that such gaps do not represent missing core sections; a complete core was recovered from each borehole. Nor can the gaps result from ablation; there is no ablation at the drill site at present and most gaps occur in the Wisconsin part of the record when temperatures were much lower than present.

Variations in vertical strain rate, and thus in annual layer thickness, over short horizontal distances, can probably explain most of the disturbances in the lowest part of the record. The strain rate is expected to vary as the ice moves over an uneven bed; thinning and 'pinching out' of horizontal layers of bubbles observed in vertical sections of core, provide evidence of such variations. The apparent gaps in the record may be highly compressed regions where the isotope variations have been smoothed out by diffusion. Faulting or movement along narrow zones of intense shear, provide another possible explanation of the gaps. Although these processes might occur near bedrock bumps and hollows, the cores show no definite

crystallographic evidence for them. Large changes in grain size occur over short vertical distances but there are no thin bands of very fine-grained ice characteristic of shear planes. Folds might also be expected to form. These would produce inverted sections of record; segments 25 and 20 in core 73 (Fig. 3) may perhaps show this. Again, however, there is no crystallographic evidence of folding.

Climate and ice thickness changes 0–5,250 BP

The point, 13 m above the bed, dated at 5,250 BP where the correlation between the two records starts to break down corresponds closely to the limit of the accurate time scale. We therefore present the results in two parts: (a) the period 0–5,250 BP

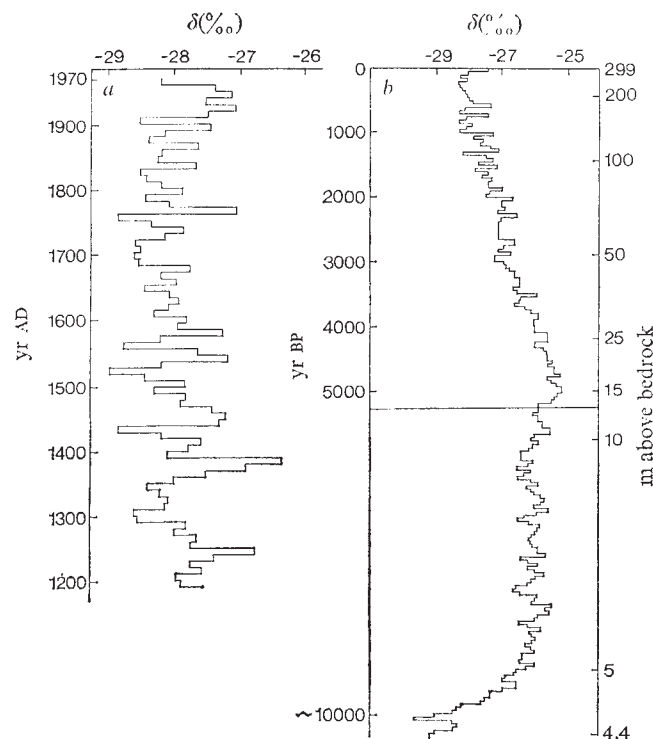


Fig. 4 *a*, 10-yr mean δ s for past 800 yr. Values from both cores are combined. The standard error of each δ is about 0.15‰. *b*, 50-yr mean δ s for past 10,000 yr. Values from both cores are combined. Beyond 5,250 BP the time scale is unreliable and there may be small gaps in the record.

and (b) the section from 5,250 BP to the bottom of the core (dated at roughly 125,000 BP by comparison with the Camp Century record) where we do not assign dates and discuss only major features. Except for the lowest 5 m, plotted δ values are an average of the values for the two cores. The estimated standard error of an annual value in the combined record is 0.5‰.

Figure 4a shows 10-yr mean δ values from AD 1200 to present. Prominent features are brief warm periods with peaks at 1240 and 1380, cold peaks at 1430, 1520 and 1560, the 'Little Ice Age' continuously cold from 1680 to 1730 and with another temperature minimum at 1760, a pronounced warming at about 1910 with relatively warm temperatures until about 1960 and a marked cooling thereafter. The Camp Century record² also shows most of these features, although some dates are different; in particular, the coldest part of the Little Ice Age occurs at about 1660. Such differences are not surprising; the Little Ice Age does not even appear in the period 1500–1700 in the record from Crête in central Greenland¹².

Figure 4b shows 50-yr mean δ values for the past 10,000 yr. The warmest part of the Climatic Optimum occurred on Devon Island at about 5,000 ± 800 BP. The date 5,000 BP is more likely

to be too old than too young because a value greater than 5060 BP would be outside the limit of 2 s.e. of the ¹⁴C date (3860 ± 600 BP) at this point.

The record shows a long-term decrease in δ from 5,000 BP to present; this does not appear in the Camp Century record^{2,4}. Figure 5 shows this feature. In *a*, curve 1 is the last 5,000 yr of the Camp Century record, smoothed by a 5,000-yr low-pass filter applied to the last 10,000 yr of the record. The time scale is that calculated from the Dansgaard-Johnsen flow model⁶. Curve 3 is a similar curve for Devon Island. Part of the difference between the two arises because the deeper layers in the Camp Century core were formed at higher elevations inland from the station. Curve 2 is the trend corrected for this effect, based on the present surface configuration and ice velocity at Camp Century and the assumption that a 100-m increase in elevation changes δ by -0.62‰ (ref. 13). The Devon record needs no correction because the boreholes are near the ice divide, provided that the divide has not migrated during the past 5,000 yr. Even after the flow correction is made, the change in δ at Camp Century is less than at Devon Island (0.6 compared with 2.2‰). On the other hand, the shorter-period trends in δ at the two stations seem to be of about the same size (Figs 5b and c).

Although it is possible that the long term cooling has been much greater at Devon Island than at Camp Century, changes in ice thickness provide a more likely explanation of the difference. Koerner¹⁴ has shown that the percentage of ice layers in the Devon cores is highly correlated with the ice cap's mass balance. He concluded that accumulation balanced ablation from about AD 1300 to 1860 and that the mass balance has been slightly negative since then. Thus during the Climatic

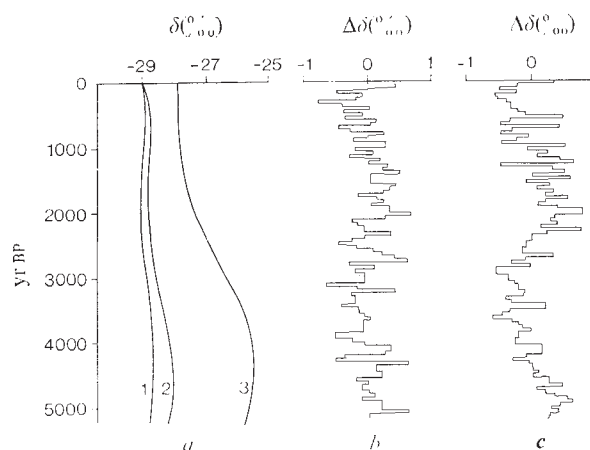


Fig. 5 *a*, Trend lines in δ for past 5,000 yr (1) for Camp Century, (2) for Camp Century corrected for ice flow from up slope, (3) for Devon Island. *b*, residual from trend for Camp Century. *c*, residual from trend for Devon Island. The Camp Century time scale is that of ref. 6.

Optimum, when δ was more than 2‰ less negative than at present, the ice cap was probably smaller and thinner than it is now. The change in δ at Camp Century would correspond to a cooling of roughly 1°. The additional 1.6‰ at Devon Island would correspond to a thickness change of about 250 m. This seems excessive in view of the broad agreement between the time scale calculated from the present vertical velocity and the ¹⁴C dates. Thus the cooling trend over the past 5,000 yr has probably been more than 1°. If so, it has been partly obscured in the Camp Century record by thinning of the ice there since the Climatic Optimum¹⁵. Other interpretations of the difference between the long term trends in δ are of course possible. Moreover, we have ignored the possibility that δ has been affected by changes in atmospheric circulation patterns.

The glaciation record

In Fig. 6a, the pre-Holocene record is a speculative reconstruction using the lowest few metres of both cores. The following rules were used in the construction: (1) Where segments of the two cores seem to be roughly equivalent the longer of the two is used. (2) Any repeated sections are used only once. (3) Where there is a large gap in one core, the record from the other core

that the Wisconsin ice originated at about 1,300 m higher than the Holocene ice¹⁶. Such a difference is more than enough to account for the exceptionally large change in δ . The most likely explanation is that the Wisconsin ice originated near the main divide of the Greenland ice sheet, whereas the Holocene ice originates within 50 km of the station¹⁷. The reason for such a drastic change in flow pattern is unclear; it may be related to the rise in sea level at the end of the Glaciation.

The difference of 8‰ between Wisconsin and Holocene ice at Devon Island should not be interpreted as purely a climatic effect because the ice probably thinned at the end of the Glaciation. A rough estimate of the difference in thickness, based on standard ice cap profiles and the assumption that the ice margin at the late-Wisconsin maximum was in the position shown in the map of Prest¹⁸, is 150 m. Because this increased load would depress the bedrock, the increase in surface elevation would be 100 m which would change δ by about 0.6‰ (ref. 13). An additional change of 0.6‰ would result from the 100 m lowering of sea level during the Glaciation. Thus the climatic effect is perhaps about 7‰. During the relatively warm period in the Glaciation δ was more negative than at present. Thus this period was an interstadial rather than an interglacial.

Oldest part of record

Except for short intervals, values of δ in the lowest 3 m of the Devon record are 0.5–3‰ less negative than at the peak of the Climatic Optimum. This segment seems to correspond to the part of the Camp Century record estimated to extend from 65,000 to 120,000 BP (Fig. 6). Values of δ are less negative than in the Climatic Optimum in much of this part of the Camp Century record also. The obvious interpretation is that, during this period, temperatures were higher and the ice thinner than at present, except for relatively brief intervals in which temperatures fell to glacial values. An alternative explanation, however, of the relatively high values of δ might be that, during this period, the major source area of precipitation on the ice caps was different from the present one. Two independent lines of evidence suggest that world sea level was below present from 65,000 to 120,000 BP (refs 19,20); thus glaciation in many parts of the world must have been more extensive than it is now. Moreover, studies of pollen²¹ and soil²² in northern Europe indicate that glacial conditions began there about 110,000 BP, although there is evidence that North Atlantic water remained relatively warm until about 70,000 BP (ref. 23). For these reasons we think that the high δ values probably indicate a different source of precipitation rather than a less extensive ice cover.

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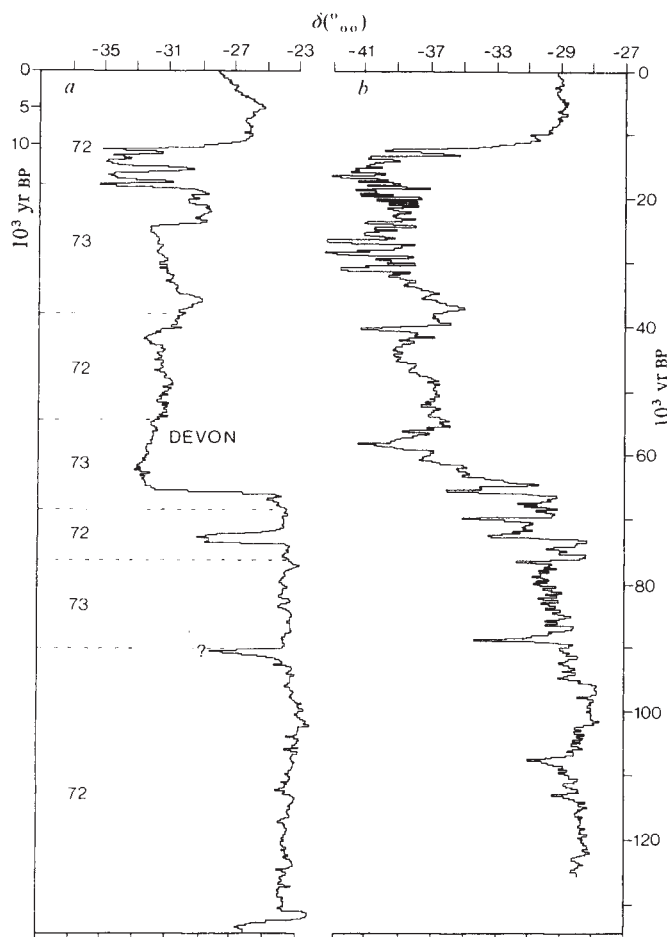


Fig. 6 a, Profile of δ for Devon Island. The Holocene section is that of Fig. 4b. The remaining section was obtained by combining the records from the lowest few metres of the two cores (See Fig. 3). While more nearly complete than either record, the combined one probably still has gaps. b, Profile of δ for Camp Century, Greenland².

is used and placed in the position consistent with adjacent common equivalent segments. (4) It is assumed that no segment is missing from both cores. (As we estimate, however, that each core lacks 25–30% of the record, it is likely that 6–9% of the combined record is missing.) The record broadly resembles that of Camp Century (Fig. 6b): a cold period between about 10,000 and 65,000 BP (Camp Century dates) preceded by a warm period of roughly the same duration.

A major difference between the stations is in the change in δ at the end of the Wisconsin Glaciation about 10,000 BP. The change at Camp Century is 11‰, at Devon Island 8‰, and 5 or 6‰ in Antarctica^{4,5}. While the temperature change at the end of the Wisconsin may have been greater at Camp Century than elsewhere, a more likely explanation is that the Wisconsin ice in the Camp Century core originated at a much higher elevation than that of the present station. Measurements of total gas content of the ice, a quantity negatively correlated with the elevation at which the ice formed, seem to support this view and suggest

letters to nature

Inverse Compton γ rays from Cyg X-3?

THE detection of ~ 100 -MeV γ rays from Cyg X-3 has been reported from recently published SAS-2 data (R. C. Lamb *et al.*¹⁷). The γ -ray luminosity is $\sim 10^{37}$ erg s⁻¹ assuming the source is ~ 10 kpc distant. Cyg X-3 is well known as an X-ray and infrared source, and a source of non-thermal radio outbursts¹ ($L_X \sim 10^{38}$, $L_{IR} \sim 10^{37}$, $L_{radio} \sim 10^{33-10^{35}}$ erg s⁻¹). The infrared, X-ray and γ -ray fluxes all display a 4.8-h modulation in phase with one another¹, which we shall presume to be attributable to binary motion^{2,3}. We note here that inverse Compton scattering of ~ 100 MeV relativistic electrons, in a region between the X-ray source and its companion, by the plentiful X rays, provides a mechanism for the γ -ray emission and its observed modulation.

Modulated γ rays are most efficiently produced by injecting the relativistic electrons in such a region for the following reasons. The γ rays cannot be created too close to the X-ray source ($R_X \sim 10^{10}$ cm), or they would be destroyed by pair creation in γ -ray-X-ray collisions. Creation outside the binary orbit would not allow sufficient modulation and would, if the emission is by inverse Compton scattering, be inefficient, as adiabatic losses would dominate the cooling of the relativistic electrons. It seems likely that the source of X-ray modulation is primarily electron scattering; the scattering optical depth must be of order unity to account for the observed amplitude of X-ray modulation (ref. 2 and references therein). But if the γ rays came from a region near the X-ray source (but $R_X \gtrsim 10^{10}$ cm), they could not be modulated by electron scattering because the Klein-Nishina cross section is greatly suppressed at γ -ray energies.

We thus have a picture of a close binary system with a cloud of relativistic electrons close to and on the X-ray source side of the primary. Modulation of the γ rays, generated by inverse Compton scattering, in phase with the X rays, arises naturally as γ rays will be most strongly emitted in backscattering X rays (compare ref. 4). The γ -ray power emitted towards the observer is approximately $\propto (1 - \sin i \cos \phi)^2$, where i is the orbital inclination and ϕ is the observed phase ($\phi = 0$ is X-ray minimum). Additional γ -ray modulation could come from partial eclipse of the emitting region as it is closer to the primary than the X-ray source, which is not eclipsed. The depth of γ -ray modulation depends upon the position and extent of the electron cloud.

What is the required source ($\gtrsim 10^{37}$ erg s⁻¹) of relativistic electrons? For a lower limit on the timescale for changes in the 4.8-h period of $\sim 10^5$ yr (ref. 5), it seems improbable that this energy could come from the orbit or the spin of the primary. The X rays themselves could drive winds at $v \sim v_{\text{escape}} \sim 10^3$ km s⁻¹ from the primary which could be shocked, but the efficiency of thus producing relativistic particles is then necessarily high ($\gtrsim 10\%$). The only remaining energy source seems to be the spin of a neutron star/X-ray source. An attractive possibility is that the source of X rays and relativistic particles is a young pulsar as suggested by Lamb *et al.*¹⁷. The X rays might then exhibit a $\lesssim 30$ -ms pulsar period. This would obviate the need for any mass transfer.

The model may be developed a little further to account for the infrared observations. If the relativistic electrons are injected in a region magnetised to say $B \sim 500$ G, then a distribution $dn_e/dE \propto E^{-4}$ for $E \geq 100$ MeV can produce the observed infrared flux. The infrared photons would be free-free

absorbed for $\lambda \gtrsim 2$ μ m accounting for the rising spectrum⁶. The observed modulation¹ can be attributed to electron scattering similar to the X rays, but with smaller amplitude as the infrared source is closer to the primary. Unfortunately, Faraday rotation in the wind would probably completely depolarise this source of infrared. We may predict, however, that if the source of γ -ray and infrared emission in this model is correct, then γ -ray and infrared variability (non-binary) should be correlated. Alternatively, if the infrared comes from a circum-binary shell as proposed by Milgrom⁷, such correlation may not be expected.

We also note that inverse Compton scattering by the stellar photons (which mostly come from incident X rays re-radiated in the ultraviolet band) and by the infrared photons in this model could account for the hard X-ray tail⁸, which would again be modulated by electron scattering. If this is correct, then the infrared and hard X-ray fluxes should show similar 4.8-h modulation and similar variability on other timescales.

Cyg X-3 is a spectacular emitter of polarised radio waves⁹⁻¹². If, as seems likely, the emission is synchrotron radiation, this provides independent evidence for the acceleration of relativistic electrons in this system. Radio emission is then detectable, however, at gigahertz frequencies only when the radio-emitting electron cloud has dimensions $\sim 10^{14}$ cm (ref. 11). Such a cloud cannot be closely related to the source of γ rays and infrared we have proposed. The radio cloud could hardly produce 4.8-h modulated γ rays, and the γ -ray emitting cloud could not expand to 10^{14} cm without suffering catastrophic energy losses. It is interesting, however, that the densities and field strengths ($n_e \sim 10^7$ cm⁻³, $B \sim 0.1$ G) proposed in a shock-wave model¹¹ for the radio cloud are compatible with those inferred above within the orbit if the field is toroidal ($B \propto r^{-1}$) and $n \propto r^{-2}$.

Finally we note that Circinus X-1, which has an X-ray period of 16.6 d (ref. 13), is also an infrared^{14,15} and non-thermal radio source¹⁶. Its light curve may be interpreted as an extreme example of the asymmetry present in Cyg X-3. A γ -ray study of this source would clearly be of interest.

Further γ -ray observation of Cyg X-3, and observations of Circinus X-1, to better define the 4.8-h light curve, is needed. Correlated γ -ray, hard X-ray, infrared, and radio observations will be extremely useful in defining the site(s) and source(s) of relativistic electron injection in this system.

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Amplification of cosmic Čerenkov radiation

EJECTION of relativistic or ultra-relativistic electrons is used in most quasar models to explain the very high luminosities of these objects. The velocity distribution of matter is taken as⁴

$$F(r) = F_0 \gamma_0^{-3/2} \quad (1)$$

where

$$F(r) = \frac{1}{M} \int_r^\infty 4\pi p r^2 dr$$

is the mass fraction external to the radius r ; $F_0 \approx 10^5$; ρ is the density and the Lorentz factor is $\gamma_0 = (1 - u^2/c^2)^{-1/2}$; u and c are velocities of the particle and the light, respectively. The observed luminosities are then explained on the basis of inverse Compton scattering of synchrotron radiation to the optical frequencies. In the radio frequency range the observed emission spectrum shows polarisation and also follows the power law, suggesting a synchrotron emission mechanism supporting these models, which essentially lead to the existence of finite magnetic fields in the quasars^{1,2}. In these conditions, some Čerenkov radiation is also produced^{4,5}. We suggest here that this radiation is amplified, and may account for the observed luminosity of quasars.

The energy density of magnetic field $B^2/8\pi$ is taken to be greater than the energy of the relativistic particles³⁻⁵ and the non-relativistic electron density, n_0 , is $< 10^8 \text{ cm}^{-3}$. The emission of synchrotron radiation at 10^8 -Hz frequency, requires a magnetic field of the order of 10^2 G. These parameters also satisfy conditions for Čerenkov emissions and may explain observed optical luminosities due to inverse Compton scattering^{4,5}. Explanations based purely on the Čerenkov emissions, however, need linear dimensions of the order 10^{14} – 10^{16} cm, which are more or less equal to quasar dimensions. Therefore, the assumptions regarding conditions for 'Čerenkov emission' in these regions are rather doubtful. Moreover, the relativistic particle distribution of the type given by equation (1) can also amplify the Čerenkov radiation, which seems to be important.

We suggest here, therefore, that the wave emitted by the relativistic particle in the Čerenkov mode might undergo amplification by Landau mechanism. It is important to point out that the Čerenkov emission and its amplification are two entirely different processes; the former is due to individual particles, whereas the latter is a collective phenomenon. Therefore, in the magnetoplasma the plasma of sharply defined energy will not amplify its own Čerenkov radiation. In the presence of a distribution of energetic particles, such processes may explain the very low frequency emission in the magnetosphere⁶⁻⁸. In the case of quasars the energetic particle distribution follows equation (1) and seems more suitable for amplification of the Čerenkov radiation, as discussed below.

A relativistic electron moving with velocity v , in a medium with refractive index $\mu > 1$, emits energy ϵ_ω ⁴

$$\epsilon_\omega = \frac{2e^2}{c^2} \omega \left(1 - \frac{c^2}{\mu^2 v^2} \right) \text{ erg s}^{-1} \text{ Hz}^{-1} \quad (2)$$

in the frequency band near ω , where e is the electron charge.

These waves, for propagation at angle θ to the magnetic field B , satisfy the dispersion relation⁶

$$\mu^2 = \frac{c^2 k^2}{\omega^2} = \frac{\omega_p^2 (\Omega_e + \omega)}{\omega \Omega_e^2 \cos \theta} \quad (3)$$

for frequencies $\omega_p, \Omega_e > \omega \gg \Omega_i$. Following Bell and Buneman, the maximum growth rate for the instability is⁶

$$\gamma_{\max} = 0.87 \left\{ \omega \left(\frac{\omega_s^2 (\Omega_e + \omega) \omega \sin \theta}{4 \omega_p^2 \Omega_e (\Omega_e \omega \cos \theta + \omega \Omega_e^2 \cos \theta)} \right)^{1/3} \right\}_{\omega = \mathbf{k} \cdot \mathbf{v}} \quad (4)$$

where $\omega = \mathbf{k} \cdot \mathbf{v}$ is the condition for resonance; ω_s , ω_p , Ω_e , Ω_i represent, respectively, the plasma frequencies of the relativistic and non-relativistic electrons, and gyrofrequencies of the electrons and ions; $\mathbf{k}$ is the propagation vector of the wave. Equation (4) shows that there is growth of Čerenkov radiation for wave propagation at a finite angle to the magnetic field. There is no growth for the angle $\theta = 0$ and 180° because for these angles there is no electric field in the direction of the wave propagation. In the case of the magnetosphere, there is not sufficient amplification of Čerenkov radiation for nearly parallel propagation because the region of amplification is very small. For quasars, however, the region of amplification can be as large as 10^{13} cm and, therefore, waves propagating nearly parallel can be efficiently amplified. The maximum growth rate for the Čerenkov radiation at frequency $\omega \approx \Omega_e$ is

$$\gamma_{\max} = 0.87 \Omega_e \left(\frac{\omega_s^2 \sin^2 \theta}{16 \omega_p^2 \cos \theta} \right)^{1/3} \quad (5)$$

If we represent the amplification factor by

$$\eta = \frac{1}{2\gamma_{\max}} \exp(2\gamma_{\max} R/V_g)$$

where V_g is the group velocity, which can be shown to be

$$\frac{2\sqrt{2} \Omega_e}{3 \omega_p} c (\cos \theta)^{1/2}$$

(from equation 3) and R is the region of amplification. For a typical optical luminosity of a quasar of $10^{46} \text{ erg s}^{-1}$ (3C 273)⁴, we obtain the relation $R^3 = 10^{27} \eta$. For an angle of propagation to the magnetic field $< 5^\circ$ and a typical value of $\gamma_{\max} = 4$, the amplification can be as high as 10^3 in the region $R = 10^{10}$ cm, which is much smaller than 10^{14} – 10^{16} cm (refs 4, 5). It is therefore possible that the optical luminosity of quasars may be due to the amplification of Čerenkov radiation.

The amplification mechanism discussed here is based on the particles having free streaming along magnetic field lines. Such particles can be scattered, however, due to hydromagnetic waves as has been discussed in the case of the galactic cosmic rays¹⁰. As this scattering affects the amplification of the Čerenkov radiation, we must include an appropriate modification in our mechanism. Detailed calculations of the characteristics of Čerenkov emission and an estimate of its role in quasar luminosities are needed. It is especially important to use the Čerenkov amplification effect correctly to account for the high luminosities of quasars.

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Measuring solar wind velocity with spacecraft phase scintillations

THE discovery of interplanetary scintillations (IPS) of natural radio sources¹ has been followed by extensive multiple-station or spaced-receiver IPS measurements of the solar wind velocity in regions not yet probed by direct spacecraft²⁻⁸. Few measurements have, however, been obtained within 40 $R_{\odot}$ because amplitude or intensity scintillations saturate when they are strong as is the case near the Sun. This is unfortunate because the scarcity of wind velocity measurements in the acceleration region close to the Sun is one of the reasons why the construction of a unique picture of solar wind dynamics has not yet been possible, despite numerous theoretical studies and direct spacecraft observations farther out^{9,10}. The demonstration of the measurement of spacecraft phase scintillations with a coherent dual-frequency radio system¹¹ paves the way for making solar wind velocity measurements with multiple-station phase scintillations. Although similar in principle to the IPS technique, several factors make this new tool a more powerful and promising one.

Because phase scintillations do not saturate when they are strong, observations can be carried out closer to the Sun than with amplitude scintillations. Spectral broadening has this same property¹² and we have obtained measurements at 2.3 GHz as close as 1.7 $R_{\odot}$ with the Helios spacecraft. Extensive phase scintillation observations in the acceleration region of the solar wind are, therefore, possible using a dual-frequency S- and X-band coherent radio link¹¹.

For multiple-station observations the baselines are restricted to distances shorter than the correlation distance of the observed scintillations. For amplitude scintillations, the correlation distance is of the order of the size of the first Fresnel zone¹³, which is roughly 140 km at 2.3 GHz. On the other hand, since the correlation distance for phase scintillations is at least 10^6 km (refs 11, 13), a much wider range of baselines is possible. Particularly suited for these measurements are the three 64-m S- and X-band tracking antennae of NASA's Deep Space Net located at Goldstone (California), Canberra and Madrid. These antennae can be used for two-station observations with baselines of the order of 10^4 km.

Because the slowly varying phase scintillations are considerably stronger than the faster varying amplitude scintillations¹³, the signal-noise ratio (SNR) needed for long baseline phase measurements is much lower than that needed for short baseline amplitude measurements. The frequency spectrum of the phase scintillations^{11,13} indicates that if the baseline distance is increased by a factor of 10 the SNR can be reduced by at least 20 dB. The choice of observing antennae for phase scintillations is, therefore, flexible, since a wide range of antenna sizes and receiver noise temperatures can be considered, in addition to the wide range of baseline distances. This is especially important since it would be desirable to add observing antennae to the group of NASA 64-m antennae to permit three-station measurements. Also, for a given SNR, slowly varying phase scintillations yield measurements at larger solar elongations than faster amplitude scintillations.

We have discussed some of the important advantages of measuring solar wind velocity with multiple-station phase scintillations. Current and future NASA deep space missions equipped with the coherent S/X radio system include Viking, Pioneer Venus and Mariner Jupiter/Saturn. Extensive observations using this new technique during the numerous superior conjunctions of these spacecraft will provide much needed

velocity measurements in the acceleration region of the solar wind. These proposed observations will complement the large number of measurements that have been and are still being made with natural radio sources and will lead to a better understanding of the dynamics and driving mechanisms of the solar wind.

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Tidal acceleration of the moon deduced from observations of artificial satellites

DOUGLAS *et al.*¹ demonstrated the existence of an apparent latitude dependence of tidal friction by determining disparate values of the second degree Love number (k_2) from perturbations of the inclinations of the GEOS-1 and GEOS-2 satellites. Lambeck *et al.*² correctly explained this phenomenon as being due to neglect of ocean tide perturbations. Parameter values for some ocean tide components have been obtained from several satellites³, but parameter values for the M_2 tide, the dominant (85%) effect of the oceans on the tidal acceleration of the Moon, have not been published. Using an improved method for computing mean elements, we⁴ obtained an observation equation for the M_2 tide from the satellite 1967-92A. Applying this technique to the satellite GEOS-3, we now obtain an additional observation equation for the M_2 tide. As shown in ref. 2, solid and fluid tide effects on satellites cannot be separated, requiring assumption of the solid tide amplitude and phase parameters for a fluid tide solution. Assuming $k_2 = 0.30$, $\delta_2 = 0^\circ$, and using the values of Lambeck⁵ for the minor O_1 and N_2 contributions to $\dot{n}_i$, our fluid tide parameters for the M_2 ocean tide yield the value of the tidal acceleration $\dot{n}_i = -27.4 \pm 3$ arc s (100 yr)⁻², in excellent agreement with the value $\dot{n}_i = -27.2 \pm 1.7$ arc s (100 yr)⁻² obtained by Muller⁶ from a combination of ancient and modern observations. These two values are lower than the value $\dot{n}_i = -35 \pm 4$ arc s (100 yr)⁻² obtained from numerical ocean tide models⁵. Our assumption of a negligible solid tide phase angle is supported by a recent determination by J. T. Kuo (personal communication) that the phase angle obtained from a transcontinental network of tidal gravimetric stations is $< 1^\circ$. Changes $\leq 0.5^\circ$ in solid tide phase angle change our result for the combined solid/fluid $\dot{n}_i$ by no more than 1 arc s per (100 yr) (ref. 2).

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Cosmic spherules as rounded bodies in space

COSMIC spherules obtained from deep-sea sediments have generally been accepted as extraterrestrial; but their mode of formation has been uncertain. On chemico-mineralogical evidence and the study of polished sections, it is difficult to refute the assumption that the spherules are merely the droplets shed from the fusion crust of a meteorite as it ablates in the atmosphere. We are now re-examining a new spherule collection (diameter > 50 μm) from surface red clay samples used previously¹; that is, Atlantic A2 (24°30' N, 64°47' W, 5949-m depth) and Pacific (22°07' S, 115°10' W, 3,060–3,200-m depth). Scanning electron microscopy of 40 'stony' spherules and 25 'irons' has led us to consider a new hypothesis—that the cosmic spherule is a round body in space and those that we find suffer only slight alteration during their grazing flight through the atmosphere. Interesting surface features exist on many of the spherules, both stones and irons, which make it difficult to believe that they have been sprayed from a meteorite. If our hypothesis can be substantiated, then cosmic spherules become important objects in their own right, a cometary origin being a distinct possibility. We now show some of these surface features and mention other supporting evidence. From the surface appearance we can classify the spherules into different types and forms, and all forms occur with similar relative frequency in Atlantic and Pacific oceans.

The 'stony' spherule type appears either as a vuggy form or an organised (near single crystal) form in about equal numbers. A vuggy form (Fig. 1), gives smooth lines of olivine and magnetite on X-ray powder photographs. The holes are suggestive of outgassing of some volatile substance during a warm stage. This example shows no sign of a fusion crust; under the optical microscope the surface is dark grey. We conclude (note the well-faceted crystals) that this spherule did not reach its melting point in atmospheric flight and was not attacked by seawater.

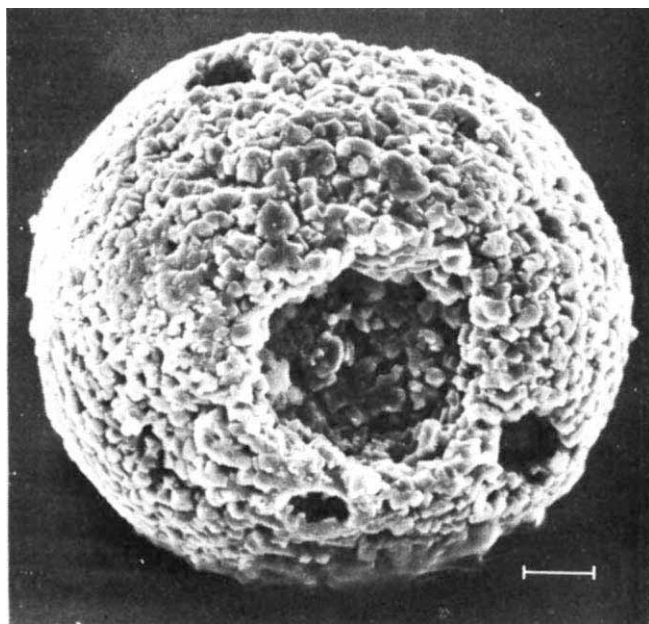


Fig. 1 A vuggy spherule. X-ray diffraction (all lines fairly smooth) gave magnetite lattice $a = 8.330 \text{ \AA}$, $\text{Fe}/(\text{Mg} + \text{Fe}) = 22\%$ for the atomic ratio of the olivine. On other vuggy spherules the crystal faces show interesting growth or etch features. Scale bar, 10 μm .

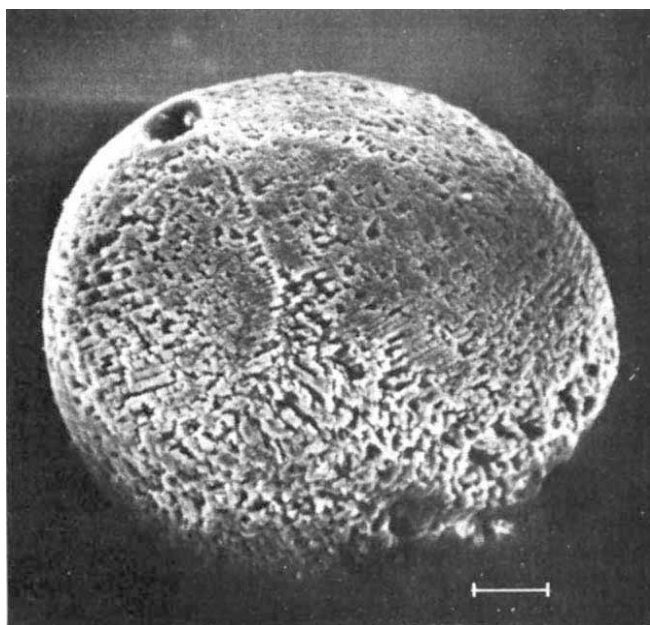


Fig. 2 An egg-shaped organised stone, uncommon because of the 'glassy' lined cupule found on the sharper pole. The spherule was polished to a diametral plane, including the cupule. The olivine crystals lie normal to the polar axis and no voids can be seen. Spot analyses of sub-micron areas gave: a somewhat variable Mg/Fe ratio; an erratic Ni distribution, which when present was $\sim 0.1\%$ Fe; and traces of Ca, Ti, Cr, Mn. X-ray diffraction (all lines very spotty) gave magnetite lattice $a = 8.356 \text{ \AA}$, and $\text{Fe}/(\text{Mg} + \text{Fe}) = 20\%$ for the olivine. From the spot analyses no large magnetite crystals were found in the interior. Part of the Ni seems to be in the olivine as well as in the magnetite. Ni in the stony phase of meteorites is < 300 p.p.m. (ref. 7). Scale bar, 20 μm .

From this and other examples, it seems that seawater has not affected our stones, collected from surface sediments. Stony spherules do erode, however; they are not found in the deeper sections of red clay cores.

Other vuggy spherules, sometimes found to be broken, show dark smoothish fusion crusts in patches. The interior is whitish and usually there is a large spherical hollow. In the unbroken state these vugs were thick-walled bubbles, with irregular-shaped holes scattered throughout the wall. X-ray microprobe analysis suggests that the magnetite is concentrated in the fusion crust. Fragile 'creamy-centred' spherules have already been described¹.

Fusion crusts on stony meteorites^{2–4} are about 100 μm thick, vesicular and very opaque. In fact there is little or no glass present (private communication). Apparently magnetite is exsolved from the melt and the melt recrystallises into an olivine with a composition poorer in Fe than the parent material. For example, a pure olivine sample ablated in a plasma jet showed a 50% decrease of Fe⁴. In some respects both vugs and organised stones are similar to fusion crustal material. We find only olivine and magnetite (also sometimes wüstite). There is a lack of glass and the vugs are vesicular. However, the distribution of $\text{Fe}/(\text{Fe} + \text{Mg})$ for the olivine in our spherules agrees with that occurring in the interior of the chondrites². If the spherules were ablation products, then the Fe content of the parent body would be greater than that commonly observed, a carbonaceous chondritic composition being more likely. But it is difficult, however, to see how vugs with whitish interiors could come from dark opaque material typical of a meteorite's crust. They seem more like micrometeorites, superficially fused or merely discoloured by moderate heating.

An organised form (Fig. 2) shows spotty olivine and magnetite X-ray lines. The texture is typical, but the presence of the 'glassy' lined cupule is very rare. In fact vesicles seem generally absent in the organised forms. The particle shown is slightly

egg-shaped with the cupule near the sharp pole. We have found one stony spherule with a globule protruding about $\frac{1}{3}$ of the way out of the surface. We mention this rare particle because it may provide the clue for explaining the cupule (Fig. 2). Perhaps during the molten stage, a magnetite globule was formed within the silicate melt and, assuming the melt was spinning, centrifugal forces would cause the globule to emerge at the sharper pole. Freezing may then flick the globule free. It could be argued that such a spinning process should occur at the moment of spraying from a meteorite's surface; and so egg-shapes, dumb-bells and even tear-drops would be a common occurrence. These forms are not found—all the spherules are near spheres.

Like the vugs, organised stones can be smooth in places. A thin fusion crust barely disguises the laminar or rod-like interior. The lack of a crust on the particle in Fig. 2 implies that it suffered little or no damage in its flight through the atmosphere. Again, seawater is unlikely to have removed a fusion crust, as the cupule is perfectly preserved. An SEM photograph in ref. 3 shows a 20- μm diameter spherule embedded in the fusion crust of Murchison. This is thought to have been sprayed off the surface of an 'upwind' piece of the shower and by chance to have been captured by the piece under investigation. This genuine ablation spherule shows a 'glassy' smooth surface. We have never found a stony cosmic spherule with such a smooth appearance, that is, having been thoroughly molten. The 'fusion' crusts on stony spherules seem very superficial. In some spherules parts of the crust have cracked away under thermal shock; in others the smoothing is only on one hemisphere and for these there is sometimes a 'brushed' appearance, strongly suggesting aligned flight through the atmosphere. Finally, the magnetite in the stones has a low lattice parameter, ranging around 8.352 Å; in the irons it ranges about a mean of 8.391 Å. Furthermore, we have never observed pyroxene in the spherules.

The 'iron' spherule type appears in two forms; those with a Ni-rich metal globule (or its oxidised remains) visible at the surface of a Ni-free magnetite-wüstite shell, and those without the metal—the oxide being only magnetite or magnetite-wüstite. A remarkable example of a metal form is shown in Fig. 3. It is the appearance of this particle that prompts us to

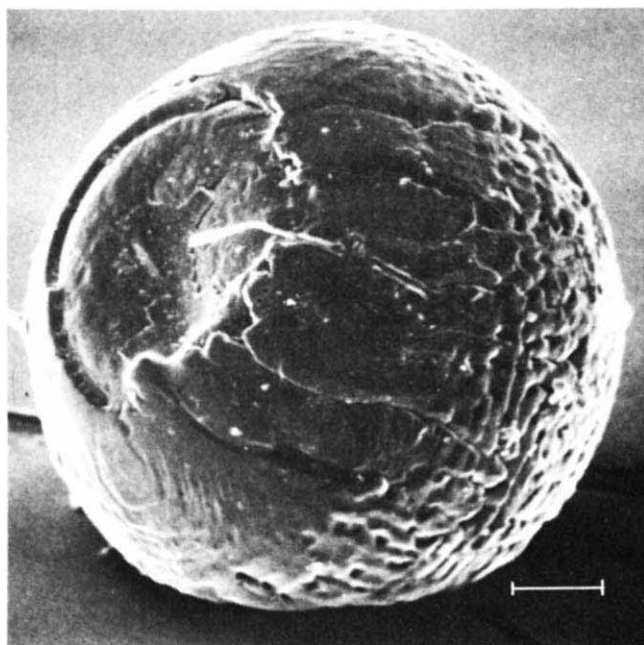


Fig. 3 A partly smooth ring structured iron—'Lucky Strike'. A polished section through the ring showed that the globule came right to the surface and had a diameter $\sim \frac{1}{3}$ that of the whole. Note the shallow depression in the centre and the fractured edges of the ring. X-ray diffraction (all lines spotty) magnetite lattice $a = 8.385$ Å, wüstite lattice $a = 4.286$ Å, alloy lattice $a = 3.573$ Å suggesting 61% Ni. Scale bar, 10 μm .

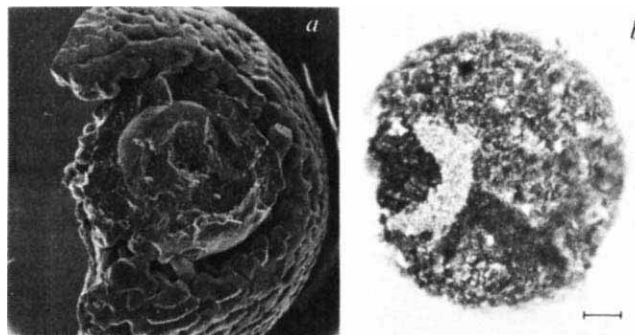


Fig. 4 A 'ballooned-iron' found as a hemisphere. *a*, the cracked-off outer part of the ballooned oxide and the small central void. Scale bar, 10 μm . *b*, An optical photograph of a polished section through the ring structure. Scale bar, 10 μm . A probe analysis for Ni gave $\sim 1\%$ in the magnetite shell, $\sim 80\%$ in metallic crescent and $\sim 30\%$ in the ballooned oxide. There is 4% Co in the metal, a trace in the shell but slightly more in the oxide scale. Spot analyses of sub-micrometre areas over the scale give Ni/Fe count ratios from 0.27 to 0.66, but many were constant at 0.55. This probably means that the oxide is nickel rich and contains in places some finely divided metallic nickel. X-ray diffraction gave magnetite (spotty lines) $a = 8.390$ Å, wüstite (spotty lines) $a = 4.280$ Å, alloy (smooth lines) $a = 3.546$ Å suggesting 82% Ni.

view all our spherules as micrometeorites. It seems to us that in space the particle was already spherical, and flight through the atmosphere must have been steady, with the heavy globule forward. The frontal hemisphere is polished, as if a film of low melting point wüstite spread from the slightly dished taenite globule. Other particles show a spectrum of damage, ranging from metallic globules that are perfect, then dished (as in Fig. 3) to markedly cupped.

Other metal forms are more complex, and we call these 'ballooned-oxides'. Here a scale grows out from the globule as it oxidises. This expansion process can crack the spherule open, and a hemispherical portion is shown in Fig. 4*a*. In Fig. 4*b*, we show a polished section. All that remains of the globule is a crescent. The ballooned oxide scale within the arms of the crescent is rich in Ni, and there is little Ni in the oxide shell. This particle is similar to one found by Finkelman (Fig. 1 in ref. 5). Foley⁶ reviewed the growth of non-molten scales on Ni/Fe alloys over a wide compositional range. For Ni $\geq 20\%$ entrapment of Ni always occurs and a nickel spinel (trevoritic magnetite) forms. We now have to account for the lack of Ni in the oxide shell and the particle's general appearance. Clearly, at one time, the particle has been thoroughly molten and in this state Ni would diffuse to the metal phase. The particle then freezes. On entering the atmosphere at grazing incidence, the temperature does not reach the melting point (note the well-faceted crystals in Fig. 4*a*) but a non-molten oxide scale balloons out, thus cracking the spherule. Magnetite scales grow on metal under water, but this cannot be the case here. We have examples of ballooned forms clearly associated with melting conditions.

In the metal forms, the X-ray lines of the magnetite and wüstite phase are spotty, but the metal lines are sometimes smooth. In every case, the low lattice parameter (around 4.284 Å) for wüstite suggests that it has grown from magnetite rather than from the metal. Furthermore, the coincident positioning of the spots on the X-ray film show that wüstite and magnetite crystals are in near-parallel orientation.

The other iron form, lacking a noticeable metal phase, can give smooth X-ray lines. The surface can appear as a 'brick-work' of near-perfectly preserved crystals. In others, however, a thin film coats the crystals in places and the crystals can be large; the X-ray lines are spotty. Both the metal and non-metal forms can have very shiny surfaces, but it seems that this does not mean that the particle has been thoroughly molten. On soaking a shiny spherule in dilute nitric acid for a prolonged period, the metal globule apparently dissolved, and the shell broke into fragments on manipulation. Large and small crystals were seen in the shell material, but the shiny surface, which

could not be resolved into crystals, is a distinct layer about 1 μm thick: a film which had spread roof-like across irregularities in the original surface. From the irons, we gain the impression that partial recrystallisation into larger crystals takes place during atmospheric heating, and if melting occurs it is only superficial.

We conclude that cosmic spherules seem to be most easily explained by assuming that they are already round in space. The spherules could come from the zodiacal cloud and those that we find have escaped vaporisation by entering the atmosphere at grazing incidence. Molten conditions must have existed at the time of their formation. Comets undergo violent eruptions and electric discharges may occur in their atmospheres. It is also possible that the refractory material within the comet is in droplet form as collisional heating could have occurred at the accumulation stage.

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Corrosion-fatigue crack initiation in metals

A DEFINITE relationship has been observed between the cyclic plastic strain-enhanced dissolution behaviour of metals in aqueous electrolytes and their corrosion-fatigue behaviour in similar conditions of environment and potential. The existence of such a

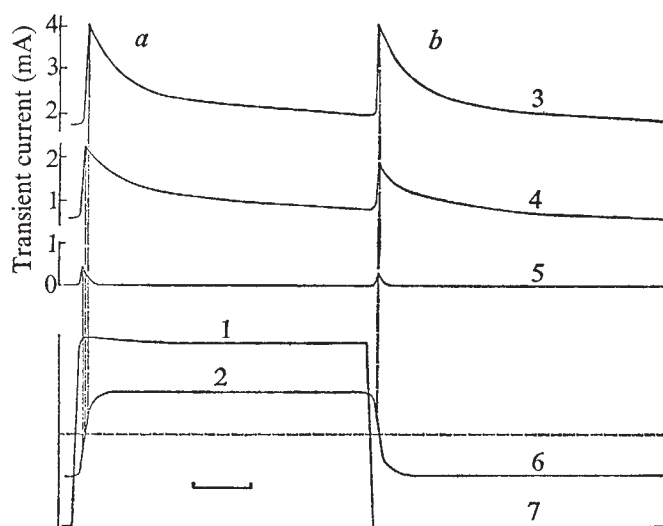


Fig. 1 Typical transients at the natural potential for mild steel in 3.5% NaCl solutions of varying pH. *a*, Tensile: 1, load -400 N mm^{-2} ; 2, strain $+0.03$. *b*, Compressive: 3, pH 1; 4, pH 8.2; 5, pH 12; 6, strain -0.03 ; 7, load -400 N mm^{-2} . Scale bar, 1 s.

relationship was first suggested in 1970¹, and the present work forms part of a wide study to examine the various effects of dynamic fatigue strains on electrode reactions at corroding metal surfaces.

The technique used involves strain cycling metal specimens under potentiostatic control in aqueous electrolytes and measuring the instantaneous electrode reaction kinetics using high-speed recording equipment. The apparatus and experimental procedure have been described in detail previously².

In the present tests a 'square' strain wave was used, giving strains of ± 0.03 about zero at a strain rate of 0.03 s^{-1} and at a frequency of 0.1 Hz. Various metals were tested in different electrolytes over a wide potential range. Tests were carried out in conditions where corrosion-fatigue failure of the metal was known to occur and in conditions where this type of failure was not observed, so that the dissolution behaviour of the metal in 'failing' and 'non-failing' situations could be compared.

Mild steel suffers from corrosion-fatigue in aqueous sodium chloride solutions of pH 1–11 but is immune to corrosion-fatigue at pH 12. Figure 1 shows $U-V$ recordings of the dissolution

Table 1 Corrosion-fatigue test and cyclic strain dissolution data for various systems

Material	Air fatigue limit (σ_a) (MN m^{-2})	Corrosion-fatigue test data		Cyclic strain dissolution data			Sign of $\frac{d\Delta I}{dN}$	Cor. fat. end. limit (air fat. end. limit) $\left(\frac{\sigma_a}{\sigma_c}\right)$
		Corrosion-fatigue endurance (σ_c) (MN m^{-2})	Test conditions material, test solution and potential	I_{peak} Values, First cycle (mA)	($\pm 3\%$ strain) 15th cycle (mA)			
0.18% C steel	250	55 (4)	0.2% C steel in 3.5% NaCl solution pH 8.2 (at the natural potential)	Tensile	0.9	1.85	+ve	0.22
		(freely corroding in 3% NaCl of neutral pH)		Compressive	0.8	1.15		
Al-Zn-Mg alloy	138	69 (5)	Al-Zn-Mg in 3% NaCl solution pH 6.5 (at the natural potential)	Tensile	70 μA	111 μA	+ve	0.5
		(freely corroding in 3% NaCl of neutral pH)		Compressive	3.5 μA	($\pm 1\%$ strain) 79 μA		
0.18% C steel	275	275 (6)	0.2% C steel in 3.5% NaCl solution pH 12 (at the natural potential)	Tensile	1.25	0.4	-ve	1.0
		(freely corroding in 3% NaCl pH 12)		Compressive	1.0	0.2		
0.19% C steel	185	194 (7)	0.2% C steel in 10% NH_4NO_3 sol. at 0.6 V (s.c.e.)	Tensile	1.0	0.4	-ve	1.05
		(in 10% NH_4NO_3 sol. at 0.6 V (s.c.e.))		Compressive	0.5	0.25		
0.42% C steel	270	270 (8)	0.2% C steel in 3.5% NaCl solution + 0.05 M Na_2CrO_4 pH 8.2 (at the natural potential)	Tensile	0.7	0.4	-ve	1.0
		(freely corroding in 3% NaCl solution + 0.05 M Na_2CrO_4)		Compressive	0.6	0.3		
Titanium	466	466 (9)	Titanium in 3.5% NaCl solution pH 8.2 at the natural potential)	Tensile	2.0	0.2	-ve	1.0
		(freely corroding in 3% NaCl solution of neutral pH)		Compressive	0.6	0.1		
Monel	265	265 (4)	Monel in 3.5% NaCl solution pH 8.2 (at the natural potential)	Tensile	0.5	0.2	-ve	1.0
		(freely corroding in 3% NaCl solution of neutral pH)		Compressive	0.2	0.12		

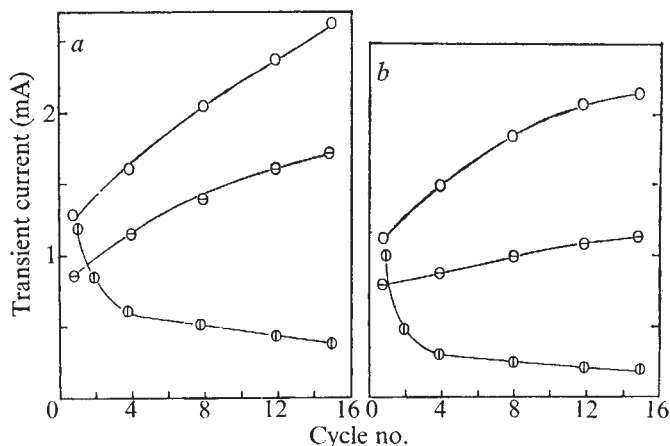


Fig. 2 The variation in the transient peak value with strain cycle number. *a*, Tensile: ○, pH 1; ⊖, pH 8.2; ⊕, pH 12.

transients produced by strain cycling mild steel in 3.5% NaCl solution of pH 1.0, 8.2 and 12.0 at the appropriate natural potentials. The transients relate to the fifteenth strain cycle. Figure 2 shows how the transient current magnitude, ΔI (defined as $I_{\text{maximum}} - I_{\text{initial}}$), varied with cycle number, N . ΔI , for the first strain cycle, was similar in all cases, but at pH 1.0 and 8.2 it increased with subsequent strain reversals whereas at pH 12 ΔI decreased progressively. Hence for the corrosion-fatigue failure situation $d\Delta I/dN$ was observed to be positive whereas for corrosion-fatigue immunity $d\Delta I/dN$ was negative. Other systems gave transients with similar characteristics, and the results are compared in Table 1 with the known corrosion-fatigue behaviour in similar conditions.

In all cases $d\Delta I/dN$ is positive when corrosion reduces fatigue life, that is when the ratio

$$\frac{\text{corrosion-fatigue endurance limit}}{\text{air fatigue endurance limit}}$$

is significantly less than one.

When the ratio equals one, $d\Delta I/dN$ is always negative. No exceptions to this rule have been found in the systems so far investigated.

The number of fatigue cycles to failure of stainless steel in sulphuric acid at different potentials was previously shown to be related to the magnitude of cyclic-strain-enhanced-dissolution effects³. It is now apparent that this principle can be successfully extended to explain the susceptibility and immunity of metals to failure by corrosion-fatigue.

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Auger electron spectroscopy of wear surfaces

THE effectiveness of lubricants containing sulphur and/or chlorine compounds in preventing wear and seizure of steels is related to the chemical lability of the load-carrying element (sulphur, chlorine) contained in them¹. This finding is consistent with the formation of surface layers of iron sulphide and chloride which prevent metal wear and seizure. Little could be deduced, however, about the nature of these protective layers. We investigate here the surface region of wear scars formed in the four-ball lubricant testing machine using Auger electron spectroscopy (AES) incorporating an argon ion sputtering technique. Here a beam of argon ions is used to etch away surface layers, and expose underlying layers for examination by AES. Using these techniques we can build up an approximate composition profile for the surface region of the

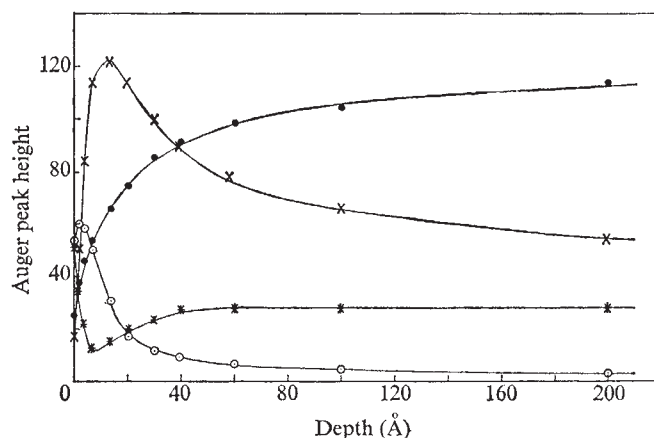


Fig. 1 Wear scar profile using dibenzyl disulphide. ●, Fe; ×, S; ○, C. The abscissa displays a depth scale in Ångströms constructed on the basis of an ion beam current density of 10^{-2} ($1 \mu\text{A cm}^{-2}$) producing a sputtering rate of 1 Å min^{-1} . This conversion is arbitrary, and takes no account of differential sputtering rates of various materials. Initial results of sputtering rates determined for pure metals has shown it to be of the right order, though somewhat too conservative.

sample. This was a more detailed investigation than that by Buckley², who used AES principally to investigate changes in the surfaces of loaded disks.

AES was carried out using a Varian cylindrical mirror analyser incorporating a 10-kV electron gun equipped with a scanning

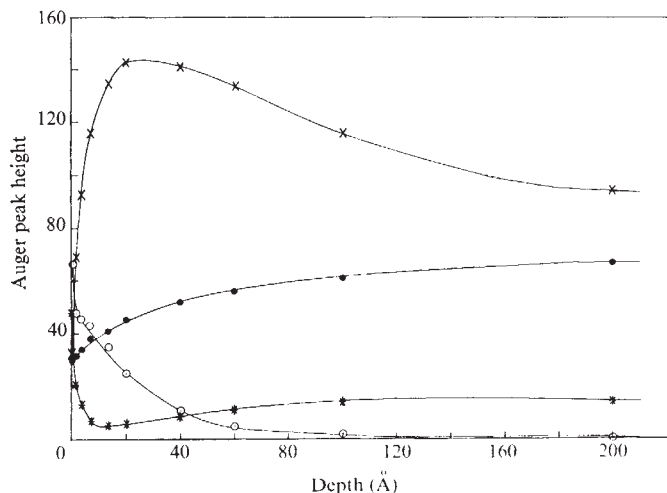


Fig. 2 Wear scar profile using di-tert-nonyl polysulphide. Symbols as in Fig. 1.

control for examination of areas from a few tenths of a square millimeter down to a focused spot of $2 \times 10^{-5} \text{ mm}^2$. We used a 5-kV beam of 10^{-6} A over an area of 0.1 mm^2 . A large area was examined to minimise effects due to surface inhomogeneity, and the low electron current density (10 A m^{-2}) reduced beam effects. A Varian 3-kV argon ion gun in a pressure of $8 \times 10^{-3} \text{ N m}^{-2}$ ($6 \times 10^{-5} \text{ torr}$) of argon was used for argon ion sputtering. The ion beam was scanned across the surface to ensure an even ion current density which was measured using a Faraday cup. Sputtering was carried out to at least 2,000-Å depth but the most important changes occurred in the first few hundred Ångströms, and this range is shown in the profiles (Figs 1–5).

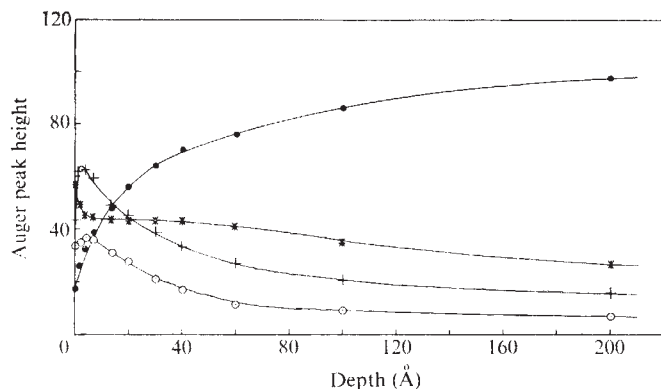


Fig. 3 Wearscar profile using Cereclor 51L. Symbols as in Fig. 1. + Cl.

We examined two sulphur-containing compounds, dibenzyl disulphide and di-*tert*-nonyl polysulphide, and two chlorine-containing compounds, Cereclor 51L (a commercial chlorinated hydrocarbon) and benzyl chloride, as lubricant additives. Blends were made up containing 15 mg-atoms of either sulphur or chlorine in 100 g of light liquid paraffin, and tested at a load of 160 kg for 1 min. The wear scars produced were washed twice in Analar toluene before examination in the Auger electron spectrometer.

The profiles obtained using the sulphur-containing oils (Figs 1

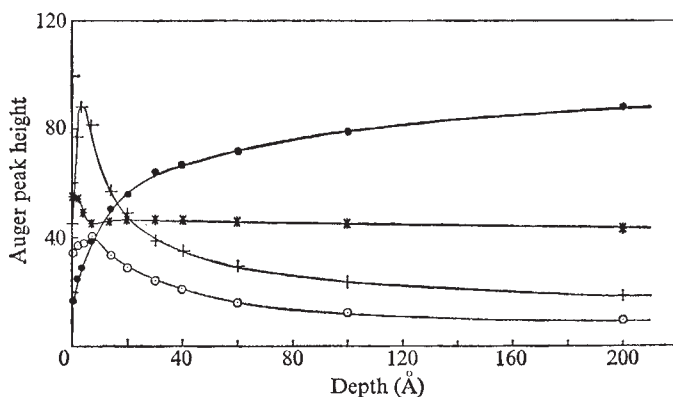


Fig. 4 Wear scar profile using benzyl chloride. Symbols as in Figs 1 and 3.

and 2) show that both oils afforded wear scars showing a high level of sulphur, presumably as iron sulphide, in the surface region. The sulphur level fell away very gradually with increasing depth, indicating considerable reaction with the metal over the first few hundred Ångströms. High levels of carbon and oxygen were present at the surface, but were greatly reduced on etching, and can probably be ascribed to atmospheric contamination.

When the scars from the balls lubricated with chlorine-containing additives were examined (Figs 3 and 4), both additives showed a high chlorine level at the surface, but the level fell away sharply on etching, suggesting that only a very thin layer of iron

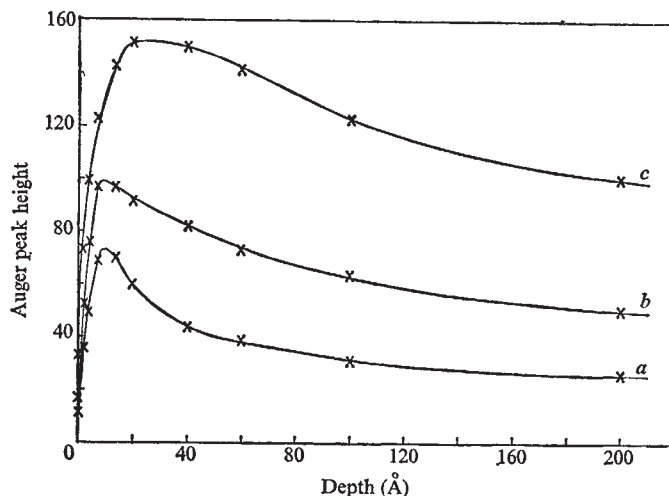


Fig. 5 Sulphur Auger profiles using di-*tert*-nonyl polysulphide at various loads. a, 50 kg; b, 100 kg; c, 160 kg.

chloride was formed. Also, a high, persistent level of carbon was found, and this was generally greater than the carbon level found with sulphur-containing oils and remaining after etching.

Certain correlations are apparent between the wear scar profiles obtained in this work and the lubricating properties of the additives. In high load conditions, the sulphur-containing oils perform better than the chlorine-containing oils as shown by the wear scar diameters: dibenzyl disulphide, 1.54 mm; di-*tert*-nonyl polysulphide, 1.48 mm; Cereclor 51L, 2.39 mm; benzyl chloride, 2.18 mm. This effect could be linked to the production of thicker layers of iron sulphide compared with iron chloride. Furthermore, comparison of the two sulphur-containing oils revealed that di-*tert*-nonyl polysulphide gives a higher maximum concentration and deeper penetration of sulphur, and is a better extreme pressure additive than dibenzyl disulphide. The improved performance of benzyl chloride compared to Cereclor 51L is consistent with the higher chlorine level at the surface produced by the more reactive compound.

As the pressure on the bearing surfaces is one of the most critical of the parameters affecting wear, we have also examined the effect of load on film formation. The sulphur profiles (normalised to the same final iron level) obtained from wear scars formed at different loads using a blend of di-*tert*-nonyl polysulphide in liquid paraffin (Fig. 5) show that, over the range examined, both the maximum sulphur concentration and the degree of sulphur penetration increases with load. The increase in the sulphur level near the surface with increasing load is obviously related to the particularly good lubrication properties of sulphur-containing oils in extreme conditions.

Examination of scars produced using mixtures of Cereclor 51L and the two sulphur compounds in liquid paraffin produced profiles showing the essential features of those obtained from the individual additives. A fuller account of this work will be published shortly.

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Structural imperfection and morphology of crocidolite (blue asbestos)

ELECTRON microscopic studies of fibrous materials, including a range of chain silicates¹⁻⁶, synthetic polymers⁷ and carbon fibres⁸, have almost exclusively involved examination of the structural characteristics visible when the fibre axis is perpendicular to the electron beam. It is extremely difficult to prepare ultra-thin sections of fibrous samples such that this axis is parallel to the beam; and yet it is in this projection that the greatest insights into structural and morphological irregularities are likely to be achieved, a fact well illustrated by the high-resolution studies of Yada⁹ on chrysotile asbestos which, being less brittle than crocidolite and other amphiboles, was amenable to the normal techniques of ultra-microtomy. We present here preliminary results of a successful axial study of crocidolite (blue asbestos), the fibrous form of the amphibole mineral riebeckite (idealised formula $\text{Na}_2\text{Fe}_3(\text{Si}_4\text{O}_{11})_2(\text{OH})_2$, space group $C2/m$, $a = 0.973$, $b = 1.806$, $c = 0.533$ nm, $\beta = 103.3^\circ$). The electron micrographs reveal several novel features.

Crocidolite from Wittenoon, W. Australia, was embedded by vacuum impregnation with Araldite (CY212) resin and thin sections (~ 2 mm) were cut across the fibre axis using a diamond saw. These sections were mechanically polished to a thickness of ~ 50 μm and finally thinned by argon-ion bombardment at a glancing angle of 12° . They were examined in Philips EM300 and Siemens Elmiskop 102 electron microscopes using a double tilt stage that enabled the fibre axis to be set exactly parallel to the electron beam. With this orientation both x^* and y^* are normal to the electron beam so that the $(hk0)$ reflections generate lattice fringes which serve as means of identifying the precise disposition of each constituent fibril with respect to its neighbour and, in addition, of characterising planar and other structural faults.

Figure 1a shows a typical section, and in its schematised analogue (Fig. 1b) the profiles of the fibrils have been delineated and their relative degrees of rotation around the z axis indicated. The electron transparent regions separating the fibrils demarcate the Araldite cement, which before hardening had penetrated the fibre and separated the fibrils slightly. The overall irregularity of the fibril profiles, notwithstanding some possible artefacts due to the ion-beam thinning, is surprising, as only occasionally do the fibrillar faces exhibit crystallographic indices which

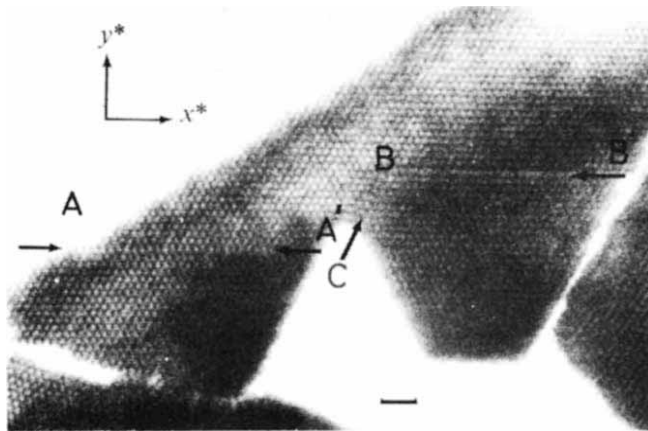


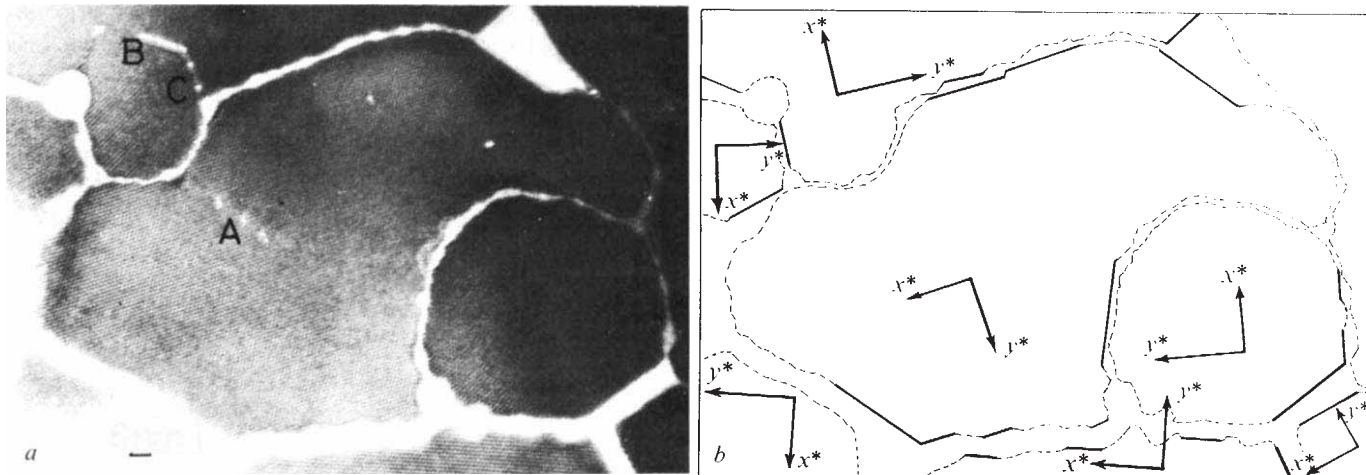
Fig. 2 Lattice image, along fibril axis, of crocidolite showing planar faults at AA', BB' (which coincide with (020) planes) and BC, which is the trace of (110). AA' and BB' are Wadsley defects consisting of triple-chains (see text). Scale bar, 5 nm.

coincide with the low-index cleavage faces (010) and (110), seen in optical studies¹⁰.

The electron-transparent regions located within the fibrils (A, B . . . in Fig. 1a) arise from isolated but aligned grown-in defects, which could well have been preferentially etched during ion bombardment. The possibility that they are micropores, incorporated during growth, cannot be discounted: the dimensions of the pores range from 2 nm (that is, corresponding to a unit-cell wide 'pipe') to over 10 nm in diameter. The alignments of these features could be the result of small-angle boundaries, for example, the (010) family may be interpreted either in terms of a tilt boundary composed of dislocations of the type $(100)[010]$ or in terms of dislocations lying on (010) slip planes with Burgers vector in the direction $[100]$.

So far as the planar faults are concerned (Fig. 2), those denoted AA' and BB' lie parallel to (020) and, by analogy with previous studies^{3,5}, it is almost certain that these correspond to triple-chain lamellae that have been incorporated into the parent double-chain matrix. They are examples of Wadsley defects^{3,5} and their presence in crocidolite is demonstrated here for the first time. At such faults there is departure from the normal chemical composition of the mineral. At the triple-chain regions a reduction in number of O atoms bonded to Si is compensated by an equal increase in the number of OH

Fig. 1 a, High resolution lattice image of ion-thinned crocidolite with the electron beam parallel to the fibrillar (z) axis. b, Schematised form of a. The reciprocal lattice directions are indicated, and the random orientation of the fibrils about their axes is apparent. —, Fibril boundaries corresponding to low-index planes. The fibrillar outlines (— · — ·) show a general irregularity.



groups. Planar faults on (110) are also present (BC, Fig. 2), the displacement vector of this extended defect, as measured on the micrograph, being $b/4$ [010]. It is noticeable that planar faults CB and BB' intersect, and the nature of the structural rearrangement that occurs in the vicinity of region B is not yet clear.

The precise significance and full characterisation of planar faults in crocidolite (as well as in other related brittle minerals which may now be studied by the technique herein described) awaits the results of dynamical calculation of lattice images^{10,11}. Such calculations are under way.

We thank the SRC and the Royal Society Exchange Scheme for support that enabled M.A.F. to work in these laboratories. We are indebted to Dr E. J. W. Whittaker for drawing our attention to this problem, and for his helpful comments on the micrographs. The technical assistance of Mr. J. L. Jenkins is also appreciated.

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Pyrolysis of a polyborodiphenylsiloxane

We have previously reported successful production of a high-strength continuous SiC fibre¹⁻³, a high flexural strength SiC sintered body⁴ and a heat-resistant Fe-Cr alloy⁵ by converting organometallic polymer polycarbosilane (PC) as the binder to an inorganic in thermal decomposition. We then found that in the thermal decomposition of organometallic polymers such as PC, an amorphous state exists before formation of the (SiC) fine particles; this SiC amorphous state is hardly possible in inorganic reactions. The process of thermal decomposition of organometallic polymers is little known. To obtain general information on the phenomenon, we have newly synthesised multi-element polyborodiphenylsiloxane containing oxygen (B, C, Si, O, H) compared with PC composed of Si, C and H, and observed the process of its thermal decomposition.

Polyborodiphenylsiloxane, (PB)p was synthesised as follows. Boric acid (0.33 mol, 20.6 g) and diphenyldichlorosilane (0.5 mol, 126.6 g) were dissolved in 200 ml of anhydrous *N*-butyl ether. The solution was then refluxed in N_2 gas atmosphere for

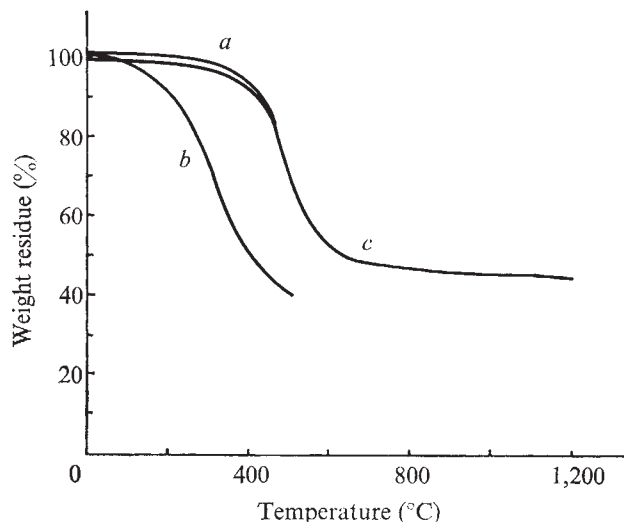
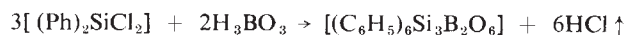
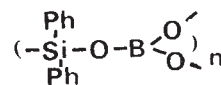


Fig. 1 Thermal analysis curves of (PB)p and (PB)m. *a* and *b*, TG curves of (PB)p and (PB)m respectively, up to 500 °C from room temperature in air. TG curve of (PB)p up to 1200 °C from room temperature in a stream of N_2 gas.

18 h, and heated for 1 h under reduced pressure at 300 °C, successively. 102 g of (PB)p was obtained as 92% yield. The (PB)p synthesis display is represented by the following reaction



The intermediate product, $[(C_6H_5)_6Si_3B_2O_6]$, is pyrolysed at 300 °C. Microchemical analysis values of the product (PB)p are shown in Table 1, with the expected theoretical ones as $[(C_6H_5)_6Si_3B_2O_6]$. The observed analytical values of the (PB)p, C and O contents are reduced in comparison to the theoretical ones and the difference can be attributed to the pyrolysing process at 300 °C of the $[(C_6H_5)_6Si_3B_2O_6]$. The softening point is 100 °C. Its infrared spectrum shows peaks at 3600 cm^{-1} (free OH), 3500–3100 cm^{-1} (hydrogen-bonded OH), 1600 cm^{-1} (C_6H_5), 1500–1300 cm^{-1} (B–O) and 1150–1000 cm^{-1} (Si–O), respectively. The product was found to be (PB)p. It is considered to contain partial structures as follows



Of the various organometallic polymers, boron polymers are generally heat resistant but not very moisture proof⁶. Vale reported the production of polyborodimethylsiloxane (PB)m from boric acid and dimethyldichlorosilane⁷. He improved its moisture-proof property by special means of electron irradiation. The (PB)p synthesised was found to be far more moisture proof than (PB)m. When both (PB)p and (PB)m were left in air at room temperature for 1 week, the transparency of the (PB)p was not changed, but the (PB)m was appreciably devitrified.

Simply by using diphenyldichlorosilane instead of dimethyldichlorosilane, we obtained here a superior moisture-proof characteristic of (PB)p facilitating practical handling. The thermal decomposition process of (PB)p and (PB)m was compared by means of a thermobalance. Figure 1 shows thermal

Table 1 Composition (wt %) of polyborodiphenylsiloxane, (PB)p heated 1,000 °C and (PB)p heated 1,500 °C

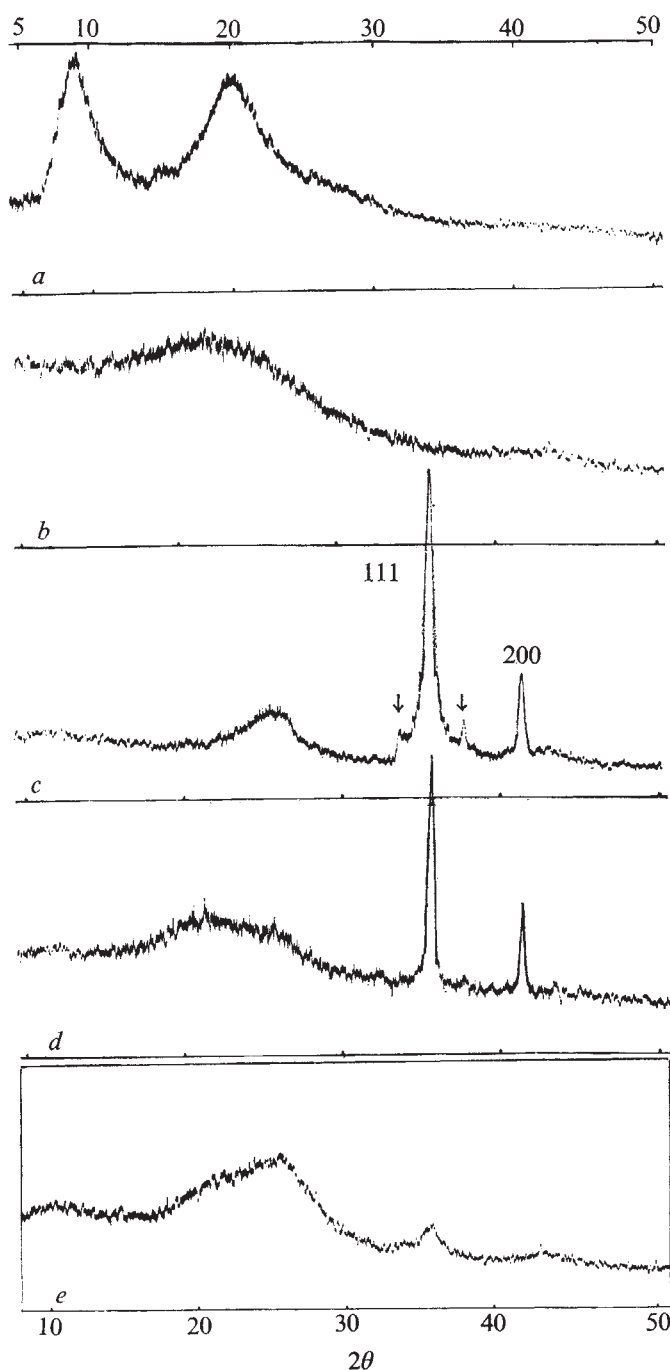
Sample	B	Si	C	O	H	N
(PB)p: experimental	3.68	16.23	59.1	7.8	4.9	< 0.2
theoretical*	3.26	12.68	65.06	14.45	4.55	—
Heated (1,000 °C) (PB)p	5.46	37.52	(~44)	12.2	0.5	< 0.2
Heated (1,500 °C) (PB)p	5.08	44.8	35.6	12.2	0.7	1.5

*The theoretical value is calculated as $[(C_6H_5)_6Si_3B_2O_6]$.

analysis curves of (PB)p and (PB)m from room temperature to 500 °C in air. TG analysis of (PB)p was examined up to 1,200 °C, in a stream of N₂ gas, and the TG curve was almost same as that of air up to 500 °C. The thermal decomposition initiation temperature of (PB)p is about 150 °C higher than that of (PB)m, indicating its superior heat resistance, and the weight loss is also smaller. (PB)p was thus greatly improved in both heat resistance and in its moisture-proof characteristic.

In pyrolysed (PB)p, the organic group is decomposed. At 1,000 °C, an amorphous phase consisting of many elements is obtained (Fig. 2b). Analytical values of the pyrolysed product

Fig. 2 X-ray diffraction patterns (CuK α) of (PB)p and the pyrolysed (PB)p products at respective temperatures. a, Polyborodiphenylsiloxane; b, 1,000 °C heat-treated (PB)p in a stream of N₂ gas; c, 1,000 °C product heated at 1,700 °C in Ar gas for 1 h; d, 1,700 °C product heated at 1,400 °C in air for 2 h; e, 1,000 °C product heated at 1,400 °C in air for 2 h. *111, 200*: diffraction peak in β -SiC.



at 1,000 °C, and 1,500 °C in N₂ gas atmosphere are shown in Table 1; and the residual as decomposition product at 1,000 °C is about 46%. The amounts of SiC of 1,500 °C sample should be at least 37 at%, based on the following assumption: all of oxygen atoms react with silicon atom and the remaining silicon atoms react with carbon and convert to silicon carbide. By further pyrolysis of the product up to 1,700 °C in Ar gas flow, phases of abundant β -SiC, glassy carbon and slight B₄C appear (Fig. 2c). In Fig. 2c, diffraction peaks (arrow) were few and weak, but they coincided with ASTM card, 6-0555 (B₄C) and the infrared spectrum of 1,700 °C (PB)p was coincided with that of a B₄C compound. The crystalline product due to the peaks is considered to be a B₄C compound.

Though constituents of (PB)p are many (including Si, B, C and O), on pyrolysis in a reducing atmosphere, its products contain mostly a β -SiC product. This phenomenon is important.

The 1,700 °C product was then burnt at 1,400 °C in air. In the X-ray diffraction, in addition to the β -SiC pattern, broader pattern than that at 1,700 °C appears between 20–16° and 30° (Fig. 2d). The compound B₄C is considered to be composed and converted to amorphous B₂O₃. The broad pattern is probably ascribed to the glassy phase of B₂O₃ and the amorphous (PB)p residue and glassy carbon.

On the other hand, when the product at 1,000 °C is further burned at 1,400 °C in air, amorphous-like SiC and an amorphous phase of multiple elements occurs (Fig. 2e).

Because pyrolysis at up to 1,700 °C produces SiC, B₄C, and so on, it is easy to convert (PB)p into a heat-resisting inorganic material. There is thus a possibility of using (PB)p as a binder in the SiC body, for example; this will be described in another report.

In conclusion, in both previously developed polycarbosilane and newly-developed polyborodiphenylsiloxane of multiple elements, there is an amorphous state during thermal decomposition to such heat-resisting inorganic compounds as SiC and B₄C. Therefore, the production of an amorphous state in the thermal decomposition of organometallic polymers to inorganics seems to be common.

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SiC and Si₃N₄ sintered bodies with new borodiphenylsiloxane polymers as binder

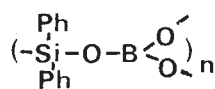
HIGH-STRENGTH SiC and Si₃N₄ sintered bodies have been studied for their application as high-temperature structural materials¹. A high-strength SiC sintered body using an organometallic polymer as the binder has been reported². A feature of this method is that polycarbosilane (PC) as the binder is converted into fine-particle inorganic β -SiC by heat-treatment, which fills the grain boundaries of the matrix SiC. In this way, with a hard-to-sinter material such as SiC or Si₃N₄, a sintered body with excellent mechanical strength can be produced at relatively low temperature

Table 1 Sintering conditions and properties of the sintered bodies

Matrix	Step	Conditions	Apparent density (g cm ⁻³)	Flexural strength (MN m ⁻²)	Fig.
SiC	a	Heating at 100 °C h ⁻¹ , keeping at 1000 °C for 1 h; N ₂ gas flow	2.55	80	a
	b	Rapid heating, keeping at 1700 °C for 1 h; 1.8 MPa Ar	2.43	109	b
	c	Rapid heating, keeping at 1400 °C for 40 h; in air	2.49	182	c
Si ₃ N ₄	a	Heating at 100 °C h ⁻¹ , keeping at 1000 °C for 1 h; N ₂ gas flow	2.13	62	
	b	Rapid heating, keeping at 1500 °C for 1 h; 1.8 MPa Ar	2.06	71	
	c	Rapid heating, keeping at 1000 °C for 40 h; in air	2.18	125	

without using a hot press. We recently synthesised a particular form of polyborodiphenylsiloxane (PB)p, an organo-metallic polymer, which is very effective as a binder. Using this binder, SiC and Si₃N₄ sintered bodies of high mechanical strengths could be successfully obtained. These results are outlined here.

A partial structure of (PB)p used as the binder is shown as



The method of synthesis properties of (PB)p and phenomena in pyrolysing (PB)p are described elsewhere³. The purity and mean particle size of both the α -SiC and the β -Si₃N₄ used as the matrix materials were 99.9% and 30 μm , respectively. The procedure giving the best result in a sintered body is as follows. About 10% by weight of (PB)p is added to the SiC or Si₃N₄ powder. To this mixture, benzene is then added, to dissolve the (PB)p. The result is a uniform mixing of binder and matrix powder. The solvent is evaporated off, and a green body of size 10×30×4 mm³ is prepared by mould-pressing at 196 MPa. The subsequent sintering process, in three steps, is shown in Table 1.

The flexural strength of the sintered SiC and Si₃N₄ bodies at room temperature was measured by the method previously described². The measured results and the densities are shown in Table 1. Scanning photomicrographs of the fractures after the successive steps of sintering are given in Fig. 1.

From X-ray diffraction after the successive steps of sintering of a SiC body (Table 1, Fig. 1), the following conclusions emerged. In step (a), the amorphous binder, not yet well crystallised surrounds the SiC particles. In step (b), the (PB)p binder has now been mostly converted into fine-particle β -SiC. Between 1,000 and 1,700 °C, flow of the amorphous binder would occur, resulting in good wetting of the matrix α -SiC. The flexural strength of the sintered body thus becomes larger than in step (a). X-ray diffraction at this stage showed Bragg peaks due to small amount of B₄C and amorphous carbon, as well as β -SiC. In step (c), the B₄C peaks disappear and the diffraction intensity indicating an amorphous phase increases. B₄C has thus become a glassy material by reaction with oxygen in the air. This glassy substance acts as a binder for the matrix SiC powder. Possibly because of this, the flexural strength of the sintered body is further raised. Similar results were obtained with the Si₃N₄ sintered body. Therefore, the sintered SiC and Si₃N₄ bodies using (PB)p as the binder will be safely usable at high temperatures and even in an oxidising atmosphere.

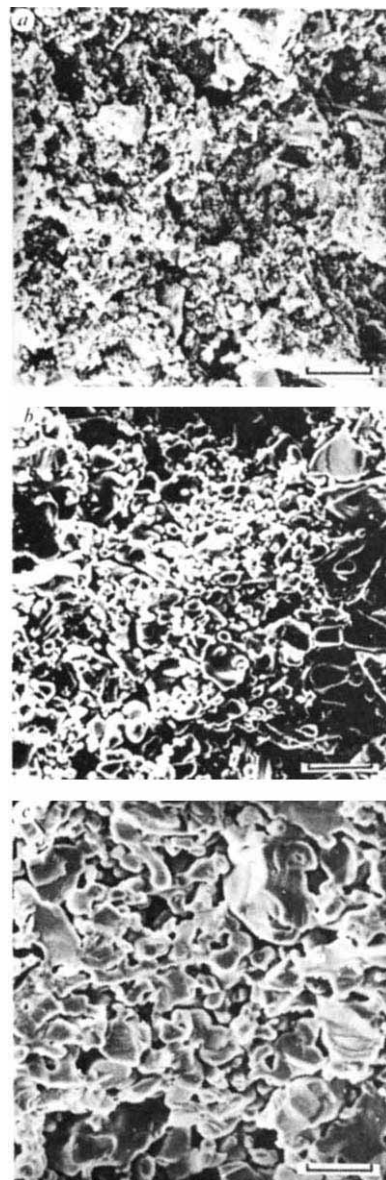
As seen in Table 1, apparent densities of the sintered bodies are relatively low. By impregnation of the sintered body with (PB)p, followed by further heat-treatment, therefore, the apparent density and the flexural strength could both be further increased. The method is similar to that described elsewhere². By applying this process to the SiC and the Si₃N₄ sintered bodies following step (a), the increases in density and strength per impregnation were

about 0.09 g cm⁻³ and 60 MN m⁻², respectively, in each case.

Compared with PC, (PB)p can be more easily synthesised and is more effective as the binder for obtaining high-strength sintered bodies.

In the SiC and Si₃N₄ sintered bodies using (PB)p as the binder, there is no dimensional change on sintering. The method of converting (PB)p to an inorganic as the binder is useful, and original, in industrial production. The sintered bodies, which are of high strength, should find many applications in various fields.

Fig. 1 Scanning photomicrographs of the fracture surfaces of SiC sintered bodies. Scale bars, 50 μm .



We thank Mr T. Amano for scanning electron micrography work.

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Production of 'prompt' nitric oxide and decomposition of hydrocarbons in flames

The pollutant nitric oxide originates in combustion systems from nitrogen in the air or the fuel. Two processes have been identified whereby atmospheric N_2 produces NO. The Zel'dovich¹ mechanism has the rate-determining step $O + N_2 \rightarrow N + NO$ and is affected by oxygen atoms having concentrations^{2,3} above those for equilibrium. Fenimore's 'prompt' scheme⁴ is an attack on N_2 by some hydrocarbon fragment with a lifetime of several μs in the flame's reaction zone⁵. Probable⁶ primary steps are



The oxidation of HCN and CN to NO has been discussed elsewhere^{6,7}. We have burnt premixed $H_2-O_2-N_2$ flames and measured the 'prompt' NO resulting from trace additions of hydrocarbons to the burner supplies. We found that 'prompt' NO yield is proportional to the number of carbon atoms in the hydrocarbon molecule. We consider that the hydrocarbons fragment into species with one carbon atom, in, or close to, the preheating region. Thus formation of 'prompt' NO, soot, ions in flames, and CH radiation from hydrocarbons all show related behaviour.

We studied flames with temperatures of 1,900–2,350 K, burnt at atmospheric pressure on a Padley–Sugden⁸ burner. They have a flat reaction zone and burned gases (diameter ~ 15 mm; length ~ 0.2 m), in which the velocity, temperature and composition profiles are those for plug flow. Without additive these flames are invisible, but with, say, 0.1% (by volume) of C_2H_2 , a greenish-blue reaction zone is apparent 1 mm off the burner face. In certain conditions this reaction zone of the hydrocarbon burning in the $H_2-O_2-N_2$ flame lifts off the burner, and to avoid this the fastest burning hydrocarbons with up to three carbon atoms, that is, CH_4 , C_2H_2 or CH_3CCH , were used. The addition of 1% of a hydrocarbon and removal of a little H_2 does not affect⁹ the temperature or concentrations of H, OH or O. Flames were sampled through a water-cooled quartz probe into a chemiluminescent analyser to determine [NO] along the axis of the burned gases. These flames were too hot for NO_2 to exist in them.

Using C_2H_2 as additive (Fig. 1) the NO with no hydrocarbon can only originate from the Zel'dovich process, whereas the increase in [NO] with C_2H_2 added can be unambiguously ascribed to the 'prompt' mode of production⁶. In general, with hydrocarbon present, [NO] near the reaction zone is non-zero, and well downstream in the burnt gases all the curves of Fig. 1 are parallel, with the increments in [NO] being 'prompt' NO. The dependence of this 'prompt' NO yield on $[N_2]$ has been

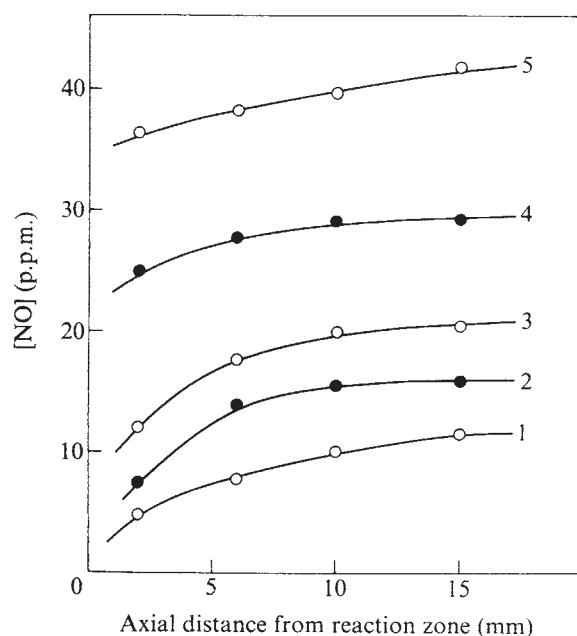


Fig. 1 Measured concentrations of NO along the axis of a flame ($[H_2]/[O_2]/[N_2] = 3.0:1.0:3.3$; temperature = 2,350 K) with various amounts of acetylene added. 1, 0%; 2, 0.98%; 3, 3.02%; 4, 4.43%; 5, 6.08%.

investigated by replacing some N_2 with argon, but maintaining the temperature constant. This has established that 'prompt' $NO \propto [N_2]$ in the unburnt gases. A similar investigation demonstrated that the yield of 'prompt' NO is also proportional to the quantity of hydrocarbon present (Fig. 2). The linearity held irrespective of the hydrocarbon, as well as from 1,900 to 2,350 K and for fuel-rich and fuel-lean flames. The only exception appeared with cooler fuel-rich ($T \sim 1,900$ K, $[H_2]/[O_2]$ in unburnt gases > 5) flames, where plots as given in Fig. 2 did show initial linearity followed by a decrease in slope for hydrocarbon additions greater than 1%, giving a maximum and a subsequent drop in 'prompt' [NO]. This suggests that hydrocarbons can destroy NO, as well as produce it.

The efficiencies of CH_4 , C_2H_2 and C_3H_4 at generating 'prompt' NO were compared (Fig. 3) for additions less than 1%. It is clear that the 'prompt' [NO] per N_2 and per hydro-

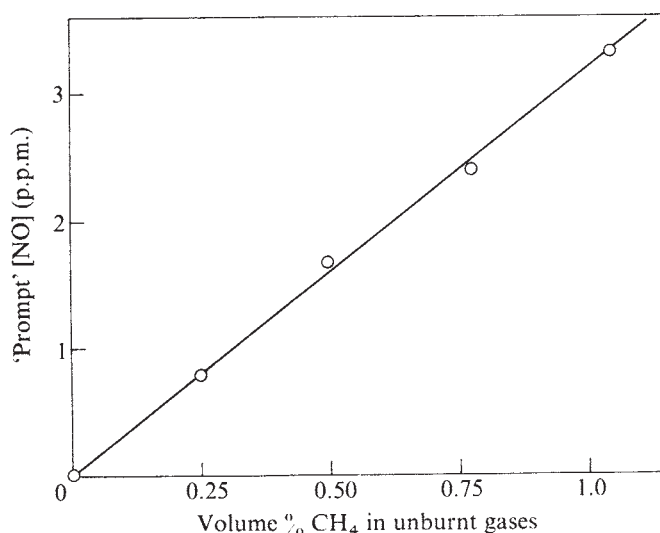


Fig. 2 Dependence of the yield of 'prompt' NO adjusted for any change in $[N_2]$ on the amount of methane added to a flame ($[H_2]/[O_2]/[N_2] = 3.0:1.0:5.7$; temperature = 1,900 K).

carbon molecule is proportional to the number of carbon atoms in a molecule of the additive. This was found to hold at different flame temperatures and compositions for fuel-rich systems. In fuel-lean flames the linearity was lost, with C_2H_2 more efficient per carbon atom than CH_4 or C_3H_4 . A separate comparison between propyne and propane confirmed that both are equally effective promoters of 'prompt' NO in fuel-rich flames. Little variation of the 'prompt' NO (per carbon atom and per N_2 molecule) was detected with temperature or $[H_2]/[O_2]$ in the unburnt gases, provided the latter exceeded two. In contrast, the 'prompt' NO in O_2 -rich flames decreased strongly for $[H_2]/[O_2]$ below two; when $[H_2]/[O_2] = 1$, there was always negligible 'prompt' NO. These observations establish that 'prompt' NO in fuel-rich systems is proportional to the number of carbon atoms present and independent of their origin. This rules out reaction (2), and others involving species containing two or more carbon atoms, as the primary step, because it is unlikely that say C_2 could come from CH_4 half as efficiently as from C_2H_2 . Reaction (1) is apparently the most likely⁶ primary step involving species with one carbon atom.

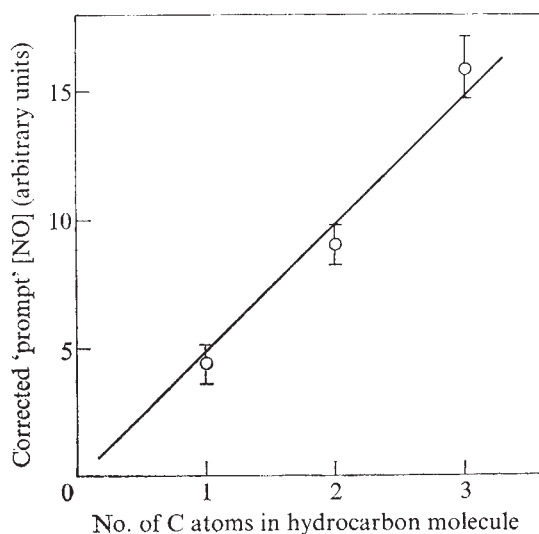
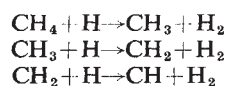


Fig. 3 The 'prompt' NO produced in a flame ($[H_2]/[O_2]/[N_2] = 3.0/1.0/3.3$; temperature $\sim 2,350$ K), per hydrocarbon molecule and per nitrogen molecule present in the burner supplies, plotted against the number of carbon atoms in the hydrocarbon.

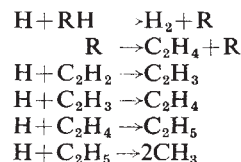
The unburnt gases are known to pass through a pre-heating region before entering the reaction zone^{5,10}. There is also an appreciable flux of hydrogen atoms into this region, because of their large concentration gradient in the reaction zone^{2,9,10} and their immense diffusivity. One explanation then of the above is that CH arises from hydrocarbons undergoing a sequence of first-order decomposition reactions, with a common rate-determining step. If this holds for CH_4 and other hydrocarbons, the rate-determining step could be the conversion of CH_3 to CH_2 or CH_2 to CH. Also, since a large hydrocarbon gives as much CH per carbon atom as CH_4 , the rupture of every carbon-carbon bond must occur before oxidation in the reaction zone interferes by producing stable products such as CO. A complete break-up of a hydrocarbon to CH_3 or CH_2 is possible given a significant inflow of hydrogen atoms and a pre-heating⁵ of less than 1000 K. Now all the stripping reactions



are close to thermoneutral¹¹ and are exothermic if OH replaces H. Also



is exothermic by some 97 kJ mol^{-1} , so that a rate-determining step cannot be identified. In fact, the reverse of each of the above reactions is likely to be fast enough at say 800 K with the large $[H_2]$ for them to be partially equilibrated and a limited 'pooling' of CH_3 , CH_2 , CH and C to result. Of course, all these radicals are likely to react with O_2 rapidly enough to prevent a complete balancing of the above four reactions. As for the pyrolysis of a paraffin in the presence of H atoms, schemes are available¹² indicating that the likeliest products include CH_4 , C_2H_4 and C_2H_2 . Such schemes, with the sequence



give the partial pool of species with one carbon atom, rapidly and first order in the hydrocarbon, before significant oxidation to CO occurs.

Since the rate constant of (1) is known¹³, a crude check on the above conclusions is possible. Consider a flame with 1% of C_2H_2 and 45% of N_2 in its supplies. If 1 in 100 carbon atoms produce CH, which is taken to react in the preheating region every collision with O_2 , then the 'prompt' NO is calculated to be 4.6 p.p.m. for the products of (1) going to NO. This compares with the observed 4.4 p.p.m. for 0.98% of C_2H_2 in Fig. 1. Such agreement indicates that the fragmentation of each fuel molecule into a pool of species with one carbon atom before significant CO production occurs is a real possibility.

This conclusion for fuel-rich flames is borne out by other work. Ferguson¹⁴ concluded that soot formation is preceded by dissociation of every C-C bond. Bulewicz and Padley¹⁵ confirmed that ions in flames originate¹⁶ from $CH + O \rightarrow CHO^+ + e^-$ with an efficiency proportional to the number of carbon atoms in a fuel molecule. Blades¹⁷ reached the same conclusion and found the CH emission intensity to be equal on a per carbon atom basis for several hydrocarbons. Peeters *et al.*¹⁸ also observed a first order dependence for the CH emission from various hydrocarbons including CH_4 .

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Lower Cretaceous Lepidoptera

LEPIDOPTERA are rare in the fossil record and, until relatively recently, most fossil butterflies and moths had been found in Tertiary deposits. Records of Lepidoptera from earlier in the fossil record have been discounted¹. The first evidence of a Cretaceous lepidopteran was Mackay's description² of the head of a caterpillar in amber (about 72 Myr BP). Kühne described micropterigid scales from Cretaceous resin (about 100 Myr BP) from West France³, and several lepidopterous specimens have been found in Canadian and Siberian ambers of Cretaceous age (personal communication from A. Mutuura and A. Skalski). There is doubt about a much earlier record reported by Riek⁴, who described two insects from Triassic beds in South Africa and placed them in the Paratrachoptera, which he considered a suborder of the Lepidoptera. (Evidence to suggest that this material is not lepidopterous will be published elsewhere.) Thus the four moths described here, which were found in Lebanese amber dating from at least 100 Myr BP, are the earliest indisputable lepidopterous specimens.

The amber was received from Professor Aftim Acra in Beirut. It was considered Aptian⁵ or Neocomian⁶ but must be at least 100 Myr BP, and Schlüter⁷ has suggested an absolute age of 130 Myr BP. The moths (Fig. 1) have a wingspan of about

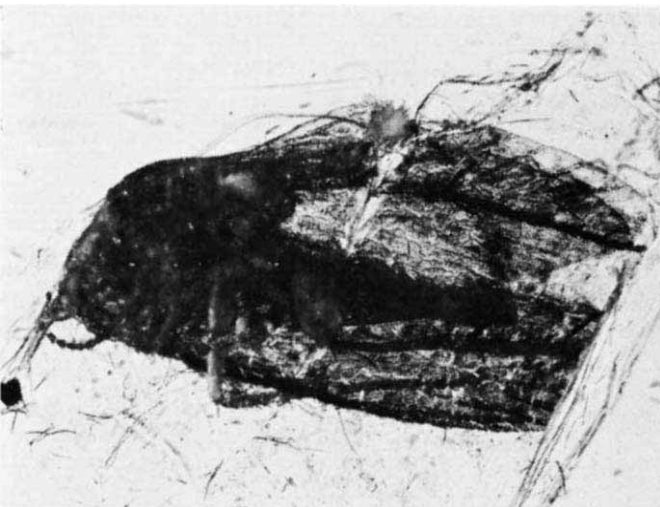


Fig. 1 Lower Cretaceous resin with lepidopterous inclusion. (Photo: British Museum Natural History.)

10 mm and have proved to be similar to extant species of Micropterigidae (Lepidoptera, Zeugloptera). They have been compared with recent species of Zeugloptera and with the primitive Daconophya, Agathiphagidae. The fossil moths have similarly shaped wings and wing scales, with abundant microtrichia on the wing membrane, comparable with recent micropterigids such as *Sabatinca* and *Micropterix*. The mouthparts have mandibles but no proboscis, and maxillary and labial palps similar to those of *Sabatinca* and *Micropterix*. Differences between the fossil and living species include the lack of ocelli and the apparent absence from the fossil forewing of a branch of the subcostal vein. The fossil moths have scales and an epiphysis on the foretibia, both of which are typical lepidopterous characters. Although the jugum cannot be seen in the fossils there is a small frenulum with four bristles similar to that of recent *Sabatinca*. A study of recent micropterigids shows that the fossil species is close to *Sabatinca* with which it has more characters in common than with *Micropterix*.

Re-examination of the holotype of *Micropterix pervetus* Cockerell⁸ from Burmese amber of Miocene age (about 7 Myr BP) shows that this species should be transferred to *Sabatinca*. Similarly *Micropterix proavittella* Rebel⁹, described from Baltic amber (Eocene/Oligocene, about 40 Myr BP) should also be

transferred to *Sabatinca*. The genus *Sabatinca* has extant species in Australia, New Zealand and New Caledonia and its former distribution and origins can now be traced back through Burma in the Miocene, Northern Europe in the Eocene/Oligocene to probable origins in the Lower Cretaceous in the Lebanon. The Australasian species of *Sabatinca* are relicts of a former much wider distribution.

The newly discovered fossil provides evidence of Lepidoptera in the Lower Cretaceous but, because of its relatively specialised morphology, I propose an earlier date for the separation of the Lepidoptera from the Trichoptera-Lepidoptera stem-group than the Cretaceous origin suggested previously. We should now be looking for fossil evidence of Lepidoptera in the Jurassic. A fuller account of the morphology and relationship of these Lower Cretaceous fossil moths will be published elsewhere.

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Photoperiodic termination of diapause in spider mites

TERMINATION of diapause in the spider mite *Tetranychus urticae* is considered to depend solely on a period of chilling, the so-called cold rest¹⁻³. The minimal period of chilling required to reactivate a certain percentage of the overwintering population may vary between populations of different geographic origin⁴. No unambiguous relationship, however, has been established between chilling period and latitude, for there may be appreciable differences in the minimal period of cold rest between strains from close localities⁵. The situation seemed more complex after Dubynina's⁶ discovery that reactivation of a Russian strain of this species increased linearly with time when mites had been induced to diapause under the influence of long days, abrupt temperature changes and severe competition for food, while reactivation was cyclical when diapause had been induced by short days and relatively low temperatures. In the latter case maximal percentages of reactivating females were obtained after diapausing mites had been kept in cold storage for 40 and 75 d. Minimal percentages were obtained after 50 and 95 d. Reactivation was complete by about the 105th day of storage. Experiments in England, however, produced no evidence for cyclical reactivation in British strains of *T. urticae*⁵. Using a Dutch strain of *T. urticae*, which has a constant photoperiodic reaction and has been reared in the laboratory for more than 15 yr (ref. 7), we have now found that termination of diapause in this species depends not only on a period of chilling but on photoperiod as well.

Female mites were induced to diapause at 18 °C in a short-day photoperiod of 10L:14D and then stored at 4 °C, in either a long-day regime of 16L:8D or a short-day regime of 10L:14D. At regular intervals samples of about 50 diapausing mites were retrieved from this cold storage and their capacity to terminate diapause was tested by placing them on fresh bean leaves at three different temperatures (Fig. 1). Females were considered to have reactivated if, within 10 d, their colour had changed from the bright orange-red of the overwintering form to the yellowish-green of the 'summer' form, and feeding and oviposition had started. Photoperiod was constant during this test; mites which had been stored in the cold in the long-day regime were reactivated in the

same long days, and mites which had spent their cold rest in short days were reactivated in short days of the same length.

The results (Fig. 1) show the influence of temperature on reactivation. With increasing temperatures during reactivation, the duration of the cold rest necessary to terminate diapause in a certain percentage of the test population becomes progressively shorter. Comparison of Fig. 1a with Fig. 1b, however, shows that at least during the first 2.5 months of diapause photoperiod had a far greater influence on reactivation than did temperature. For example, at 17 °C 50% of the mites terminated diapause in long-day conditions after a cold rest of only 8 d, but in short-day conditions termination of diapause in 50% of the test population was achieved only after a cold rest of 54 d. This difference in duration of cold rest, required to terminate diapause in 50% of the mites in the two photoperiodic regimes, is still 34 d at 20 °C and 28 d at 23 °C (Fig. 1). About 2.5 months after the beginning of diapause the photoperiodic sensitivity of diapausing females had apparently disappeared, for reactivation of the complete population succeeded from that moment onwards in short as well as long days. Thus termination of diapause in *T. urticae* is dependent not just on temperature, but, at least during the first few months, on photoperiod as well.

These experiments suggest that sensitivity to photoperiod remains in adult females of *T. urticae*, at least during the first few months of diapause, and that in long days and relatively high temperatures diapause can be terminated in part of the population without the intervention of a cold rest of even minimal duration.

Although even in the short-day regime, at all three temperatures tested, complete reactivation was achieved after a cold storage of about 70 d, experiments in both photoperiodic regimes were prolonged up to 120 d to look for any cyclicity in the pattern of reactivation. Reactivation proved to be linear, however, in

accordance with the results obtained with British strains of *T. urticae*⁵, and in contrast to Dubynina's⁶ results.

Our results were obtained with a laboratory strain of *T. urticae*, and experiments with strains isolated from different localities in Europe are in progress to check the generality of the phenomenon of photoperiodic termination of diapause in this species. Some speculation, however, is possible about the ecological significance of the sensitivity of diapausing mites to photoperiod. It is conceivable that sensitivity during the early days of diapause tends to retain the diapause state during the autumn, when occasional warm days might break diapause in part of the population. According to our results the short days of autumn would prevent this breaking of diapause, while during winter diapause would be sustained and prolonged by prevailing low temperatures. In this sense, photoperiod would not function to terminate diapause when external conditions are favourable again, as in several insect species, but rather to protect the mites from becoming active again long before the season favourable for development and reproduction.

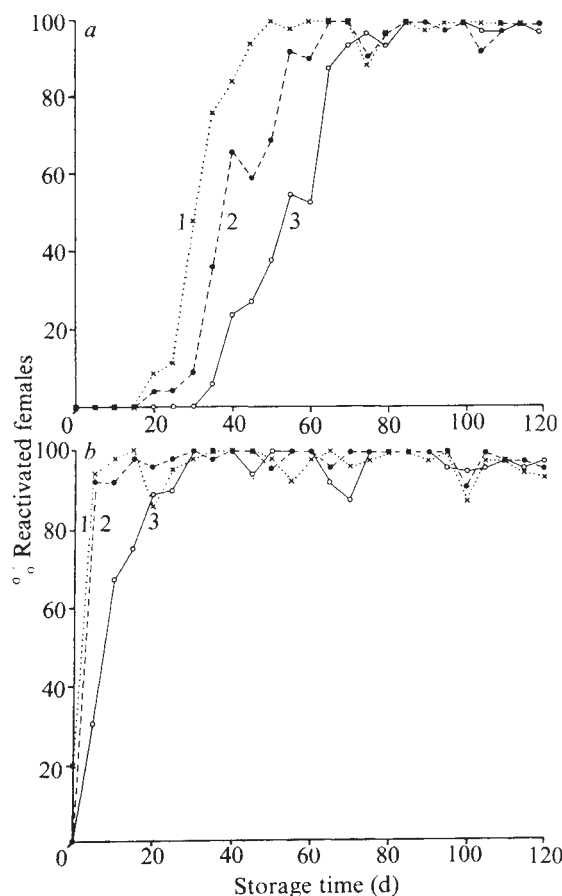
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Fig. 1 Reactivation of diapause females of *T. urticae* at three different temperatures (1, 23 °C; 2, 20 °C; 3, 17 °C) in relation to the cold rest during which the mites were stored at 4 °C. a, Cold rest and reactivation in short days (10L:14D); b, cold rest and reactivation in long days (16L:8D).



Albinism in relation to competition in bamboo *Phyllostachys bambusoides*

THE ecological consequences of albinism in plants have not been investigated, although the phenomenon has been considered by physiologists, geneticists and cytologists^{1–7}. Production of albino offspring generally reduces parental reproductive value, because albinos die after seed reserves are depleted, and thus do not reproduce. This negative effect can be moderated if a recessive allele for albinism confers increased fitness in the heterozygous state^{8–13}. I present here a hypothesis that, in species producing dense stands of seedlings subject to inter-specific competition, albino offspring can confer a competitive advantage to photosynthetic siblings; the reduction in parental fitness due to albinism may thus be less than predicted on the basis of reduced number of offspring. A test using bamboo (*Phyllostachys bambusoides*) supports this proposal.

Production of albino offspring by a given parent at a given time could increase growth and survivorship of non-albino members of the same cohort if the albinos shade interspecific competitors, but impose less intense intraspecific competition than would the same number of photosynthetic seedlings. As albinos deplete their seed reserves and die, adjacent photosynthetic seedlings should expand their crown and root systems to fill the space. Shading of competitors is thus maintained. Decomposition of albinos will release nutrients which can be absorbed by surrounding plants. The parental investment of resources in albino seeds may therefore be regained in part by photosynthetic offspring. The amount regained will depend on intensity of interspecific competition, as well as on rate of nutrient leaching from the soil.

This hypothesis was tested experimentally on Barro Colorado Island, Panama (9°10'N latitude, 79°51'W longitude, 50 m elevation). Green and albino seedlings of *P. bambusoides* Sieb. and Zucc. (Graminae), a monocarpic bamboo introduced from Asia, were grown alone and in competition with seedlings of *Sida acuta* Burm. f. (Malvaceae), a very widely distributed pantropical weed. The bamboo occurred as a dense clone approximately 5 m wide by 10 m long, with stalks of approximately 15 m. The bamboo was located on the edge of a large

Table 1 Mean dry weight per individual and percentage mortality for experimental plants

	1	2	3	4	Treatment no.		7	8	9	10
Number of plants per pot*					5	6				
Albino bamboo	0	0	0	0	0	24	48	48	0	0
Green bamboo	24	48	96	24	48	24	0	48	0	0
<i>Sida</i>	0	0	0	48	48	48	48	0	48	96
Green bamboo†										
Mean dry weight ($\text{g} \times 10^{-4}$)	212	206	142	164	146	203	—	196	—	—
95% confidence limit	14.7	13.4	9.0	20.9	12.8	21.5	—	21.3	—	—
% mortality	6	8	10	8	13	0	—	11	—	—
<i>Sida</i>										
Mean dry weight ($\text{g} \times 10^{-4}$)	—	—	—	70	71	67	75	—	78	64
95% confidence limit	—	—	—	6.6	5.4	5.0	6.6	—	6.8	6.0
% mortality	—	—	—	0	2	45‡	3	—	3	19

* 24 plants per pot — 1,323 plants per m^2 ; 48 plants per pot = 2,645 plants per m^2 ; 96 plants per pot = 5,290 plants per m^2 .

† Median tests for green bamboo dry weights (d.f. = 1 in all cases): treatment 1 vs 4, $\chi^2 = 7.66$, $P < 0.01$; 1 vs 6, $\chi^2 = 0.01$, not significant; 4 vs 6, $\chi^2 = 9.46$, $P < 0.01$; 1 vs 3, $\chi^2 = 11.06$, $P < 0.001$; 2 vs 8, $\chi^2 = 0.46$, not significant; 3 vs 8, $\chi^2 = 14.70$, $P < 0.001$; 3 vs 6, $\chi^2 = 11.44$, $P < 0.001$.

‡ 65% in one replicate, 25% in the other; median test for green bamboo dry weights within treatment 6, replicate 1 vs replicate 2, $\chi^2 = 0.33$, d.f. = 1, not significant.

clearing. It produced abundant seed between January 1975 and July 1976, and then died. Dense stands of seedlings appeared around the clone during the wet seasons of these years (May–December). Average seedling density on 19 May 1976 was $1,549 \pm 1,089$ seedlings per m^2 ; $25.4 \pm 3.21\%$ of all seedlings were albino. This ratio was also demonstrated during the wet season of 1975 (Carol Augspurger, personal communication). A one-to-three ratio of albino to green seedlings suggests that albinism is determined by a recessive gene. Average leaf area per seedling on 19 May was $1.4 \pm 0.32 \text{ cm}^2$ for green bamboo, and $1.4 \pm 0.40 \text{ cm}^2$ for albinos. *Sida acuta* was the only species producing abundant seedlings of uniform size in the same habitat as *Phyllostachys* at the time of the study. It is an herbaceous perennial growing to a maximum height of approximately 2 m in clearings and on forest edges.

Seedlings of both species were excavated on 6 May 1976. Mean height of excavated seedlings was $7.2 \pm 1.15 \text{ cm}$ for green bamboo, $6.3 \pm 1.16 \text{ cm}$ for albinos and $1.3 \pm 0.19 \text{ cm}$ for *Sida*. Bamboo seedlings were approximately 2 weeks old at the time of collection, and all retained connections to seeds which seemed to contain endosperm. *Sida* seedlings were in the cotyledon stage and had not yet produced mature leaves. Seedlings were transplanted with bare roots to soil-filled clay pots 15.2 cm in diameter and 12.3 cm deep. Soil was a Frijoles clay¹⁴, collected from a site near the bamboo clone, and thoroughly mixed. Seedling densities in each treatment are given in Table 1. There were no significant between-treatment differences in height for a given type of seedling at the beginning of the experiment. Height was linearly correlated with dry weight in a series of non-experimental seedlings: for green bamboo $r = 0.81$, $P < 0.01$, $n = 44$; for albino bamboo $r = 0.88$, $P < 0.01$, $n = 44$; for *Sida* $r = 0.84$, $P < 0.01$, $n = 45$. Each experimental treatment was replicated twice. The two replicates were initiated at different times during the planting period, to avoid between-treatment differences due to time of planting. Lack of sufficient *Sida* seedlings of uniform size prevented greater replication. A carefully measured grid system was used to ensure uniform spacing of seedlings. In treatments containing more than one seedling type, seedlings were arranged in regular alternating sequences (ABAB, AABAAB, ABACABAC, and so on) determined by relative proportions of each type.

Pots were placed in a growing house with walls and roof of insect screening. A clear plastic sheet was used to eliminate rain. The growing house was in a clearing and could receive direct insulation from mid-morning until mid-afternoon. The experiment took place during the wet season, when cloudy weather was predominant. Each pot received 60 ml of untreated water from Lake Gatun every other day. Location of each pot relative to others was randomised and changed every other day to

avoid position effects. All seedlings which died during the first 2 d after planting were replaced. Replacement of albino seedlings was continued for 2 weeks after planting. Initial mortality of albinos seemed to result primarily from damage to seedling-seed connections during planting and was evenly distributed among treatments. Marked albino plants in the field did not die during this period. The experiment was terminated after 37 d, when all albinos had died. Marked albinos in the field had also died by this time. Dead seedlings decayed very rapidly due to fungal attack. Seedlings were cut at ground level, dried to constant weight at 70 °C, and weighed.

Results (Table 1) are consistent with the hypothesis. Green bamboo seedlings grown in pure culture at three densities showed lowest mean dry weight per plant at highest density. This demonstrates intraspecific competition, a necessary condition for the hypothesis. Green bamboo grown at intermediate density with an equal number of albinos, however, did not differ significantly in dry weight from green bamboo in pure culture. Thus, albino and green bamboo do not seem to compete. Green bamboo grown at low and intermediate densities in the presence of *Sida* had significantly lower mean dry weight per plant than did plants grown in pure culture at the same densities, demonstrating interspecific competition. When green and albino bamboo were grown together in the presence of *Sida*, however, mean dry weight per green bamboo plant was not significantly different from that for controls. Mortality of *Sida* seedlings was much greater in the presence of a mixed green–albino population than when grown only with green bamboo or only with albino bamboo. Differences among means for *Sida* dry weight were not statistically significant. Mortality of green bamboo was too low to permit meaningful comparisons among treatments.

This experiment provides the first evidence that plants with a lethal genotype can confer a competitive advantage to siblings with non-lethal genotypes. The primary result of this competitive effect in natural populations might be a reduction in the intensity of selection against albinism; the fitnesses of parents producing partially albino cohorts could remain lower than fitnesses of parents producing entirely photosynthetic cohorts despite the competitive effect. To be of adaptive value, the benefits of improved competitive ability would have to outweigh the energetic cost of producing seeds with albino genotypes, the cost of reduced number of viable offspring, and potentially, the cost of reduced genetic variability among viable offspring due to reduced numbers. Surveys are planned to determine if the phenomenon is likely to occur in natural populations. Regardless of its importance in natural conditions, agricultural applications of this effect should be considered.

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Figure 1 consists of four panels. Panel (a) shows three wheat spikes: B (left), BxW (middle), and W (right). Panel (b) shows RFLP gel electrophoresis results for wheat lines B, BxW, and W, with a '+' marker at the top and a '-' marker at the bottom. Panel (c) shows G-banded karyotypes of wheat lines B and BxW. Panel (d) shows G-banded karyotypes of wheat lines B and W.

A portion of the chromosome pairing in the barley-wheat hybrids would be expected to be of the autosyndetic type not unlike that observed in the haploids of both

[illegible]

species. The number of paired chromosomes in the barley-wheat hybrids, however, exceeded the sum total of paired chromosomes in haploid barley and haploid wheat (Table 1). Genotypes in diploid wheat species such as *T. tripsacoides*⁷ (*Ae. mutica*) and *T. longissimum*⁶ suppress the effect of diploidising genes in wheat to variable degrees. A mean chiasmata per cell of 1.82 in the barley $\times$ wheat hybrids indicates that the barley genotype may also suppress the diploidising system in Chinese Spring similar to that of the weak suppressors reported in *T. tripsacoides*⁷ and *T. longissimum*⁶ which showed 1.51 and 1.75 chiasmata per cell, respectively, in hybrids with Chinese Spring. The data suggest that in barley as in some diploid *Triticum* species a locus (or loci) has evolved that permits some homoeologous pairing in hybrids with unrelated species. It remains to be determined whether there are extensive intraspecific differences in the genus *Hordeum*, as there are in certain species of the genus *Triticum*, for genes that relax the suppression of homoeologous pairing of wheat chromosomes. The infrequent occurrence of a heteromorphic bivalent at metaphase I suggested that there may be some homology between chromosomes of barley and wheat, providing an alternative explanation for the increased pairing in the hybrid above the sum of the two haploids. The application of Giemsa staining techniques for meiotic chromosomes in barley-wheat hybrids could help to solve this problem.

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Detection in primitive gymnosperms of proteins and glycoproteins of possible significance in reproduction

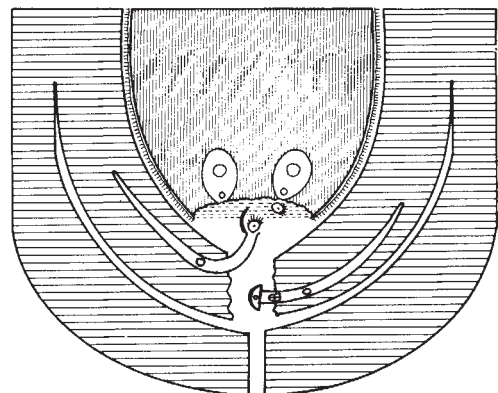
THE modern cycads are the remnant of a gymnosperm group that has been in existence for at least 200 Myr¹. Cycads are dioecious, with separate male and female plants, and fertilisation is effected by motile male gametes—so-called zooidogamous reproduction. Zooidogamy is found also in *Ginkgo*, another dioecious gymnosperm of considerable antiquity¹. Reproduction in all other gymnosperms and in flowering plants is siphonogamous. Here fertilisation occurs through a pollen tube which conveys the non-motile male gamete to the egg. A pollen tube is produced in the zooidogamous gymnosperms, but it is not involved in gamete transfer; it simply anchors the male gametophyte to the ovule during gametogenesis (Fig. 1). The ripe male gametes are released to swim freely in a pool of fluid bathing the openings of the egg-containing archegonia (Fig. 1). It is not known how the gametes that carry out fertilisation are selected in these plants. Dioecism ensures outcrossing, but there is presumably some form of intraspecific screening. The male gametophyte or male gamete could, for example, identify itself to the parent of its prospective mate, or the male gamete to the egg cell at the threshold of syngamy. I report here the occurrence in cycads of proteins and glycoproteins at sites in the pollen grain and ovule where they might be involved in reproduction.

Nonspecific esterase activity was detected in cryostat sections of *Macrozamia reidleyi* pollen slurries using 5-bromoindoxyl acetate and α -naphthyl acetate as substrates, the latter in a coupling reaction with fast blue B salt². For

electron microscopy, the pollen was incubated in the reaction mixture before glutaraldehyde fixation and osmium post-fixation. Controls were run without substrate in all tests. Acid phosphatase activity was localised in freeze-sectioned pollen by Gomori's lead salt method³ and the controls were incubated in the reaction mixture containing 0.01 M sodium fluoride. Nonspecific esterase activity was localised in cryostat sections of fresh *Cycas thouarsii* ovules by the indigogenic method. Acid phosphatase was investigated in ovules in the same way as for the pollen. Concanavalin A (con A)-binding sites in the megaspore wall of *C. thouarsii* were revealed ultrastructurally in glutaraldehyde-fixed ovules by coupling with horseradish peroxidase (HRP) and diaminobenzidine (DAB)⁴, and by fluorescence microscopy in fresh, frozen sections with con A labelled with fluorescein isothiocyanate (con A-FITC, L'Industrie Biologique Française). The con A in the controls for both methods contained 0.2 M α -D-methyl mannoside⁵. Homogenates of *C. siamensis* megagametophytes were prepared in buffered saline, filtered and dialysed against water. The dialysate was concentrated and the concentrate was tested for ability to agglutinate trypsinised, human O+ erythrocytes and analysed by sodium dodecyl sulphate (SDS)-polyacrylamide electrophoresis⁶ after dissociation of the proteins in mercaptoethanol and SDS. The gels were stained with Coomassie blue for proteins and the periodic-acid-Schiff technique for carbohydrates.

Proteins with enzymatic properties are contained in the pollen grain wall and the cytoplasm of the male gametophyte in *M. reidleyi*. The indigogenic method reveals discrete granules of nonspecific esterase activity in the tube cell cytoplasm (Fig. 2a). Electron microscopy of pollen incubated in α -naphthyl acetate shows a narrow layer of nonspecific esterase activity between the intine and exine at the sulcus of the grain (Fig. 2e). Intense acid phosphatase activity is also associated with the intine at the sulcus (Fig. 2c). The localisation of hydrolytic enzymes in the apertural intine in the cycad accords with the occurrence in the flowering plants⁷⁻⁹, where the enzymes are implicated in the penetration of the stigma¹⁰. By analogy, the cycad hydrolases could be utilised in the metabolism of substrates encountered by the pollen tube during its growth through the nucellus (Fig. 1). It is interesting that conifer pollen germinates in the pollen chamber of *Ginkgo*^{11,12} and produces tubes which enter the nucellus tissue¹³—penetration presumably being facilitated by the hydrolases detectable at the pollen sulcus⁷. This provides evidence about the regulation of mating behaviour in *Ginkgo*: the ovule

Fig. 1 Diagram of post-pollination events in the ovule of a cycad. Two pollen grains have germinated and their tubes penetrated the nucellus. Male gametes are being released from the lower grain into the fertilisation fluid held in a depression—the archegonial chamber—at the apex of the female gametophyte. The egg-containing archegonia are left unshaded and the megaspore wall is represented by a series of radial lines. The various structures are not drawn to scale.



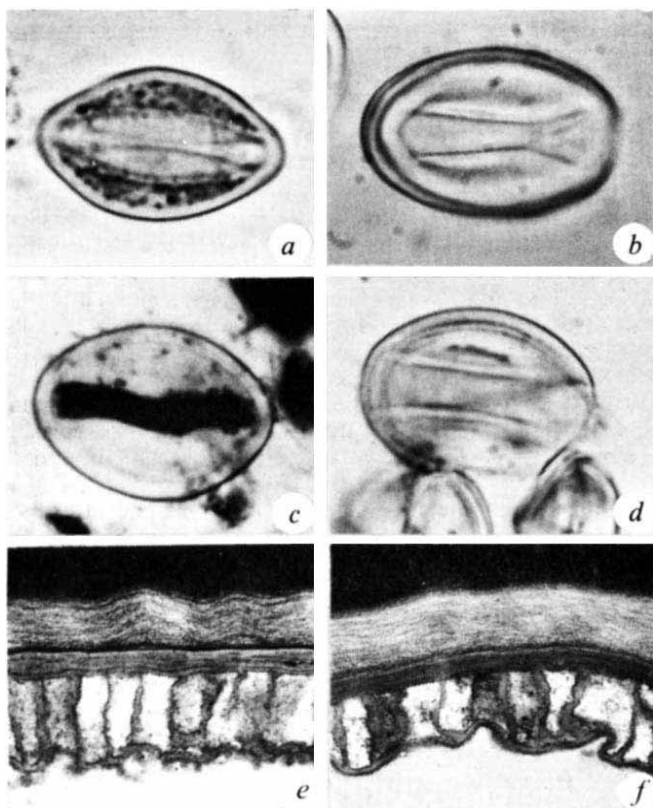


Fig. 2 Pollen of the cycad *M. reidley*. *a*, Nonspecific esterase activity ($\times 1,000$). *b*, Esterase control ($\times 1,000$). *c*, Acid phosphatase activity ($\times 1,000$). *d*, Acid phosphatase control ($\times 1,000$). *e*, Electron microscope localisation of nonspecific esterase activity between the exine and intine at the sulcus ($\times 20,000$). *f*, Control for (*e*) ($\times 20,000$).

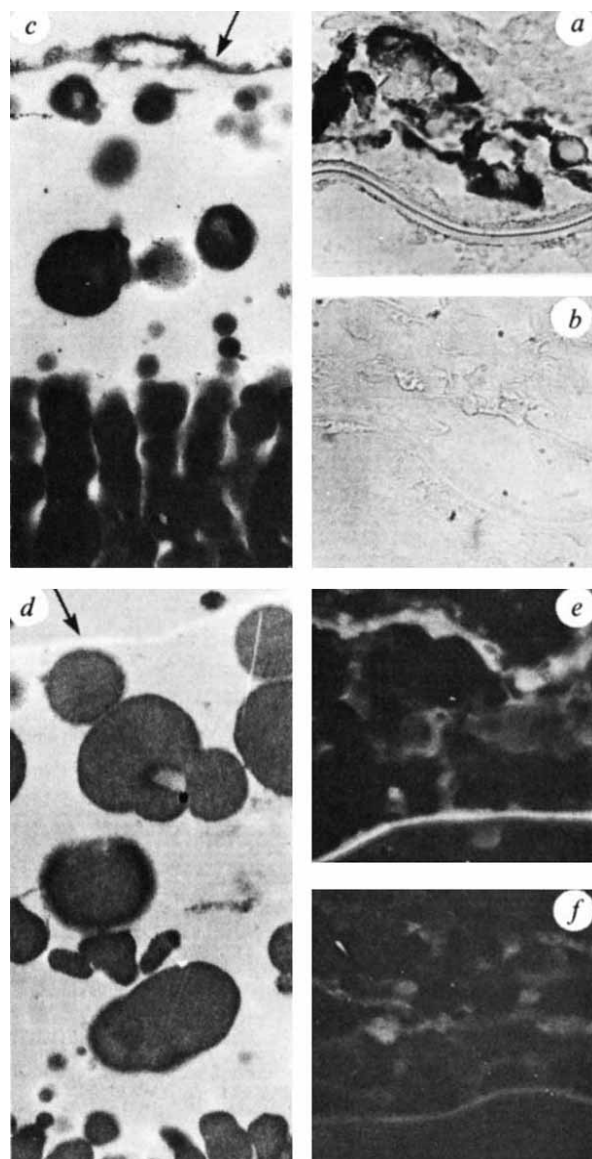
possesses no capacity for suppressing germination or frustrating the growth of the pollen tube in wide pollinations.

The female gametophyte in the zooidogamous gymnosperms is contained by a multilayered megaspore wall, comparable with the flowering plant pollen wall¹³. During development material derived from autolysis of the sporophyte cells immediately external to the gametophyte accumulates in the chambered outer layer (exine) of both walls. In the flowering plants the source cells comprise the tapetum^{10,14,15} and in the gymnosperm ovule they are the inner layers of the nucellus¹³. There is a degree of chemical conformity between the walls and sporophytic loads in the two groups. Strong nonspecific esterase activity, indubitably of sporophytic origin, is found in the residue resulting from the breakdown of the nucellus cells in *C. thouarsii* ovules (Fig. 3*a*). There is no detectable acid phosphatase activity. Esterases, but not acid phosphatase, occur in the pollen exines of many flowering plants and their activity is judged a characteristic marker for the transfer of proteins from the tapetum to the pollen grain surface¹⁶.

My experiments have demonstrated a carbohydrate moiety in the cycad megaspore wall able to bind con A. Electron microscopy shows that in *C. thouarsii* con A is bound specifically to the surface of an electron-lucent wall component incorporating sporopollenin droplets derived from the nucellus cells (Fig. 3*c*). Moderate to strong FITC fluorescence is emitted by the degenerating nucellus cells and by the megaspore exine in freeze-sectioned ovules treated with con A-FITC (Fig. 3*e*), the fluorescent material on the wall corresponding to the con A-binding layer observed in con A-HRP coupling. The specificity of con A towards oligosaccharides with terminal glucose and mannose residues is well authenticated¹⁷, and acceptor sites for it are present in the pollen wall¹⁸ and stigma surface pellicle—the receptor site for pollen-borne recognition molecules^{19–21}—in flowering plants^{22,23}. If the pellicle is coated with con A,

penetration of the stigma is prevented in compatible pollinations²³, suggesting that the con A sites are necessary to one level of acceptance response in the reproduction of flowering plants. This cannot apply in the cycad megaspore, because above the archegonia the wall is dispersed before the male gametes are released (Fig. 1). But if this process is contingent on necrosis of the female gametophyte tissue to form the archegonial chamber (Fig. 1), the fertilisation pool could hold in solution molecules from both components. A concentrated extract of megagametophyte plus wall of *C. siamensis* haemagglutinates trypsinised human erythrocytes (Fig. 4*a*), signifying the presence of a phytohaemagglutinin or lectin²⁴. Electrophoretic analysis of the extract reveals nine protein bands (Fig. 4*c*) three of which give a reaction for carbohydrates (Fig. 4*d*). In the conditions

Fig. 3 Ovule of cycad *C. thouarsii*. *a*, Nonspecific esterase activity in the degenerating nucellus cells. The megaspore wall is the refractive structure towards the bottom of the picture ($\times 500$). *b*, Control for (*a*) ($\times 500$). *c*, Megaspore wall after incubation in con A and coupling with HRP. Con A is bound to the surface of the electron-lucent wall component; the resultant DAB reaction product is indicated by the arrow ($\times 20,000$). *d*, Con A control. The white line indicated by the arrow is the surface of the electron-lucent wall layer ($\times 20,000$). *e*, Con A binding sites revealed with con A-FITC. Strong fluorescence is emitted by the degenerating nucellus cells and megaspore exine ($\times 300$). *f*, Control for con A-FITC test. The section shows only autofluorescence ($\times 300$).



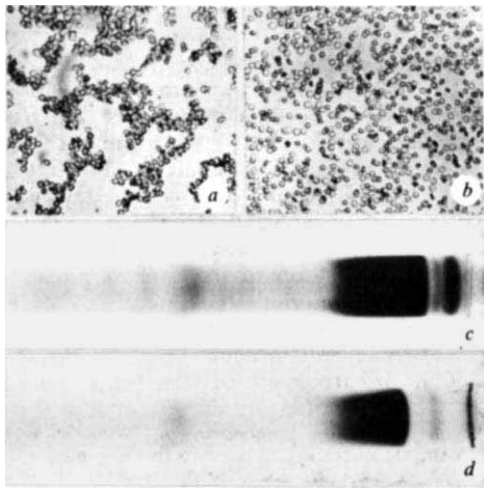


Fig. 4 Agglutination of trypsinised human erythrocytes produced by a saline-held extract of the megagametophyte and megaspore wall of the cycad *C. siamensis*. *b*, Control preparation for the agglutination test. Cells were treated with 0.15 M saline instead of extract. *c*, Protein bands in an SDS-polyacrylamide gel of the extract. *d*, Complementary gel stained for carbohydrates.

of the run these are likely to mark carbohydrates of glycoproteins.

This finding offers an intriguing possibility for the control of intraspecific mating in the primitive, zooidogamous gymnosperms. It has been suggested that lectins in plants have a defence function and may also be involved in the determination of self-recognition between cells²³. Lectins interact with animal cells²⁴ and with plant cell protoplasts²⁵ and can detect intraspecific variations in surface oligosaccharides²⁴. As a consequence of these properties a function in recognition can be envisaged for the lectin in the gymnosperm ovule: as a constituent of the fertilisation pool it could be involved in discrimination between male gametes.

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Isozymes of a tunicate and a cephalochordate as a test of polyploidisation in chordate evolution

IN view of the uncertainty regarding early phylogeny of the chordates^{1,2}, Atkin and Ohno³ determined the nuclear DNA contents of representatives of three chordate subphyla. *Ciona intestinalis* (Tunicata) has a diploid DNA content of 0.42 pg, while *Amphioxus lanceolatus* (Cephalochordata) contains 1.2 pg—very close to the 1.4 pg considered as the basic vertebrate value^{4,5}. Assuming a tunicate-like ancestry of the cephalochordate-vertebrate stock^{6–9}, Ohno¹⁰ proposed that the threefold difference in genome size between *Ciona* and *Amphioxus* might reflect the occurrence of at least one round of polyploidisation preceding vertebrate evolution. To test further this hypothesis we determined the number of genes coding for various enzymes in *C. intestinalis* and *Branchiostoma* (*Amphioxus*) *lanceolatus*. Only few genetically interpretable isozyme data from *B. lanceolatus* have been reported^{11,12}. An isozyme study on a related species, *B. moretonensis* has been published recently¹³. Systematic isozyme investigations in tunicates have not been reported before. We report here that *C. intestinalis* and *B. lanceolatus* have identical gene numbers for homologous enzymes with only one exception.

Comparative isozyme studies have proved a powerful tool in substantiating ancestral diploid-tetraploid relationships, for example in teleostean fish¹⁴ and various examples of gene duplication in mammals including man have been taken as support of the idea of ancestral polyploidy in this taxonomic category^{15,16}. Thus, if relative to *Ciona*, *Branchiostoma* represents an ancestrally polyploid organism, it is conceivable that it still possesses an increased number of functional gene loci. Table 1 summarises the results of our electrophoretic investigations. Data from 10 of the 12 isozyme systems analysed were interpretable in each, *Ciona* and *Branchiostoma*. Of these, nine lend themselves to a direct comparison: five enzymes seem to be encoded for by a single gene locus in *Ciona* as well as *Branchiostoma*; one system (AAT) is clearly encoded for by two gene loci in both organisms, whereas the number of loci underlying the multiple zones of superoxide dismutase activity remains unclear, since only the slowly migrating zone resolved into sharp bands. The NAD-MDH pattern observed in *Ciona* is best interpreted by the assumption of two loci while in *Branchiostoma* a third, additional NAD-MDH locus is present. Multiple zones of esterase activity were seen when 4-methylumbelliferyl-acetate was substrate. The dimeric enzyme seen in human tissue in these conditions¹⁷ appeared in *Branchiostoma* only.

Our data do not support the polyploidisation hypothesis of Ohno, since in one isozyme system only (NAD-MDH) *Branchiostoma* is endowed with a higher number of genes than *Ciona*. It should be emphasised, however, that our data do not contradict a polyploidisation hypothesis. In the course of regaining diploidy, a tetraploidised organism tends to functionally eliminate the duplicate genes by accumulation of 'forbidden' mutations, unless some special selection pressure favours their maintenance^{5,14,18,19}. *Branchiostoma*, if anciently polyploid, may already have silenced the majority of duplicated genes. It should be mentioned that with respect to the ribosomal DNA content, apparently subject to especially drastic change during evolution²⁰, there is a 1:6 ratio between *Branchiostoma* and *Ciona*²¹. The failure to detect more duplicated genes in *Branchiostoma* using the whole body extracts from adult animals may also be due to a possible differential activity of gene duplicates during development and/or in different

Table 1 Analysis of electrophoretic data of isozyme studies in *C. intestinalis* and *B. lanceolatum*

Enzyme		No. of animals investigated	<i>C. intestinalis</i>			<i>B. lanceolatum</i>			Allele frequencies*	
			No. of alleles found	Allele frequencies*		No. of animals investigated	No. of alleles found	Allele frequencies*		
NAD-MDH	fast	21	2	0.33	0.66		100	2	0.01	0.99
1.1.1.37	slow	21	3	0.03	0.94	0.03	100	3	0.03	0.96
	very slow						100	2	0.01	0.99
NADP-IDH		13	3	0.07	0.73	0.20	very faint staining			
1.1.1.42										
6-PGDH		9	5	0.33	0.28	0.17	very faint staining			
1.1.1.44				0.17	0.06					
Catalase		20	2?				13	3?		
1.11.1.6										
Peroxidase		no staining					13	3	0.04	0.92
1.11.1.7									0.04	0.04
Superoxide dismutase	fast	23	variable				very faint staining			
1.15.1.1	slow	23	2	0.20	0.80		13	2	0.73	0.27
AAT	fast	14	3	0.20	0.55	0.25	14	3	0.04	0.32
2.6.1.1	slow	14	2	0.10	0.90		14	2	0.04	0.96
PGM		27	2 ?				14	5	0.11	0.32
2.7.5.1									0.29	0.18
Methylumbelliferyl-esterase	fast	12	3	0.17	0.67	0.17	17	variable		
3.1.1.1	slow	12	variable				17	variable		
	very slow (dimeric)									
Adenosinedeaminase		14		unvarying			17	3	0.09	0.88
3.5.4.4							14	5	0.25	0.07
Glyoxalase I		8	3	0.06	0.81	0.13			0.07	0.07
4.4.1.5							14	2	0.18	0.82
PGI		11	6	0.05	0.23	0.05				
5.3.1.9				0.36	0.14	0.18	95	2	0.98	0.02

All animals were caught in the North Sea at Helgoland. *C. intestinalis* individuals were freed from their tunics before further preparation. All animals were homogenised in 0.01 M phosphate buffer, pH 7.4, containing 10^{-4} M $MgCl_2$, in a glass-glass homogeniser, and the 20,000g supernatants were subjected to horizontal starch gel electrophoresis. All enzymes were analysed in gels consisting of 14% starch in 0.08 M Tris/0.03 M citrate buffer, pH 8.0 (bridge: 0.223 M Tris/0.068 M citrate buffer, pH 8.0) except esterase, adenosinedeaminase, glyoxalase and superoxide dismutase (the latter in the case of *Branchiostoma* only); these enzymes were investigated in 13% starch in 0.006 M potassium phosphate buffer, pH 7.4 (bridge: 0.375 M potassium phosphate buffer, pH 7.4). Staining was performed by conventional methods^{17, 24-26}. Attempts to stain lactate dehydrogenase, glucose-6-phosphate dehydrogenase, sorbitol dehydrogenase and alcohol dehydrogenase were either unsuccessful or yielded results too poor for genetic interpretation. MDH, maleate dehydrogenase; IDH, isocitrate dehydrogenase; 6-PGDH, 6-phosphogluconate dehydrogenase; AAT, aspartate amino transferase (GOT); PGM, phosphoglucomutase; PGI, phosphoglucose isomerase.

*Most cathodal variant first.

organs^{5, 12}. Furthermore, as Ohno⁵ has pointed out, duplicated genes may have been altered with respect to kinetic properties, pH and temperature optima, and substrate specificity. Thus their detection by standard electrophoretic and staining procedures may be prevented.

An alternative to the polyploidisation hypothesis is that the common ancestor of tunicates—cephalochordates and vertebrates already possessed a DNA content similar to the basic present-day vertebrate value, and that during the evolution and specialisation of the tunicates an appreciable part of the nuclear content was lost. Loss of nuclear DNA content concomitant with specialisation has been noted by Hinegardner²².

As pointed out by Ohno¹⁰ a further alternative to the polyploidisation hypothesis relies on the assumption that the genome size increase in *Branchiostoma* relative to *Ciona* (or its ancestor) was due to accumulation of repetitive DNA. It has been shown²³ that the *Ciona* genome contains approximately 30% repetitive DNA, but the genome composition of *Branchiostoma* is not known. Its analysis might clarify chordate interrelationships.

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Similarity of the general designs of protochordates and invertebrates

THE protochordate amphioxus is clearly differentiated from the invertebrates by most taxonomic criteria^{1, 2}, and is recognised as the organism most closely resembling the early fish-like vertebrate progenitors^{3, 4}. Recently, however, Manwell⁵ has provided evidence that, with respect to enzyme variability, amphioxus resembles invertebrates rather than vertebrates. We report here that, in terms of gross sequence characteristics of DNA, amphioxus and two other protochordates, *Ascidia mentula* and *Ciona intestinalis*, more

closely resemble the DNAs of the invertebrates, echinodermata and brachiopoda, rather than the DNA of higher vertebrates (craniata).

Characterisation of the DNA was by nearest-neighbour two-base sequence (doublet frequency) analysis and subsequent determination of the patterns of deviation from random expectation of the doublet frequencies (general designs)⁶⁻⁸. The technique of doublet frequency analysis, originally developed by Kornberg and his colleagues⁹⁻¹¹, measures the frequencies of occurrence of the sixteen possible doublet sequences in DNA. Normalisation of the observed doublet frequencies to give the general design yields a characteristic pattern which has six degrees of freedom for a double-stranded DNA. Expressing doublet frequencies as patterns of deviation from random expectation removes the components of variation in frequency which are a consequence of base composition alone, leaving an underlying pattern of sequence-dependent characteristics which are independent of G+C content. This is a very stable characteristic of DNA (refs 7 and 12), and similarity in general designs in DNAs is considered to reflect past response to similar or identical evolutionary selection pressures (and is thus a measure of evolutionary relatedness). When general designs were calculated from the results of Swartz *et al.*¹⁰ for three echinoderms (2 echinoidea and 1 asteroidea) and six vertebrates (1 fish, 1 bird and 4 mammals), the echinoderm and vertebrate DNAs fell into two readily distinguishable groups while the DNAs within each group were closely similar¹².

Table 1 gives the doublet frequencies and base compositions of three protochordate DNAs from amphioxus (*Branchiostoma lanceolatum*), *Ciona intestinalis* and *Ascidia mentula*, two brachiopod DNAs from *Crania anomala* and *Terebratulina* sp. and two echinoderm DNAs from *Antedon bifida* (Crinoidea) and *Thyone fusus* (Holothuroidea). The lower craniates are represented by the DNAs of two fish; the primitive cartilaginous dogfish (chondrichthyes) and the higher bony fish, coelacanth (osteichthyes; crossopterygii). For comparison, Table 1 includes the doublet frequencies determined by Swartz *et al.*¹⁰ for the echinoderm, *Echinus esculenta* (Echinoidea) and the higher fish, salmon (Osteichthyes; Actinopterygii), the most primitive

vertebrate previously analysed. Also shown are the G+C contents gained from doublet frequency data and, where available, from buoyant density determinations. The observation that G+C contents calculated from doublet frequency data are generally slightly lower than those given by buoyant densities has already been noted^{8,13}. It is relevant that none of the DNAs was found to contain any significant proportions of density satellite DNAs. Even on close examination of the doublet frequency data of Table 1, it is difficult to come to any firm conclusions with regard to comparisons of the invertebrate and craniate DNAs. However, when the general designs (that is, functions of sequence characteristics independent of total base composition) are compared (Fig. 1), those of the protochordates are found to be very similar, not only to each other, but also to the general designs of the echinoderms and brachiopods. Like these invertebrates, the protochordates differ from the lower craniates with particular reference to the relative frequencies of the doublets AG-CT and CG.

Evaluation of the differences seen in the histogram presentation of the general designs was achieved by cluster analysis using the weighted average linkage method¹⁴ with Euclidean squared distance. This was done for the general designs shown in Fig. 1 together with those of the DNAs of the higher vertebrates: guinea pig (Mammalia), chicken (Aves) and bullfrog (Amphibia) and the echinoderms *Paracentrotus lividus* (Echinoidea) and *Asterias rubens* (Asteroidea). The resulting dendrogram (Fig. 2) clearly shows that the craniate and protochordate DNAs fall into separate groups. The protochordates are indistinguishable from the invertebrates by this method. The small differences within the protochordate-invertebrate group are not much greater than the experimental error inherent in doublet analysis¹³, and differences in undetected satellite populations could have contributed here. The larger differences seen within the vertebrates are undoubtedly real with the design remaining stable from mammalia to amphibia, but with differences becoming pronounced at, and within, the fish. Some of these differences may be due to satellite DNAs, but in the dogfish the general design for slow renaturing nuclear DNA is identical to that of total nuclear DNA (unpublished results).

Table 1

Brachiopods				Echinoderms				Protochordates				Craniates											
<i>Crania</i>		<i>Terebratulina</i>		<i>Echinus</i>		<i>Antedon</i>		<i>Thyone</i>		<i>Ascidia</i>		<i>Ciona</i>		<i>Branchiostoma</i> (Amphioxus)		<i>Dogfish</i>		<i>Coelacanth</i>		<i>Salmon</i>			
AT	104	105		91		119		87		82		94		83		70		71		73			
TA	90	92		75		109		68		70		77		71		53		62		68			
AA	TT	121	124	119	128	100	116	119	119	81	90	96	105	82	92	86	99	73	69	103	109	74	83
GT	AC	55	56	56	55	53	62	58	52	60	57	62	58	61	55	65	59	61	59	55	48	62	62
TG	CA	66	65	67	65	69	76	68	62	72	70	75	72	72	68	77	71	82	76	73	66	75	77
GA	TC	53	53	51	52	49	64	51	47	61	62	52	56	56	50	58	60	74	68	52	56	57	67
AG	CT	49	50	48	49	47	64	50	47	55	55	54	57	52	54	58	60	75	72	61	65	69	75
GG	CC	34	32	32	32	31	46	29	24	55	50	44	44	54	49	44	43	59	54	58	59	49	52
GC		27		27		35		28		41		45		39		40		40		45		40	
CG		20		20		22		18		35		30		32		28		18		18		17	
a		32.9		32.8		30.0		34.2		28.1		29.0		28.5		28.5		27.6		28.3		27.6	
t		33.3		33.9		32.4		34.2		29.3		30.5		30.2		30.6		27.1		30.0		29.3	
g		17.0		16.7		16.8		16.6		21.7		20.3		21.0		20.7		23.4		21.0		21.0	
c		16.7		16.7		20.8		15.1		21.0		20.3		20.3		20.1		21.9		20.8		22.1	
%G+C		33.7		33.4		37.6		31.6		42.7		40.5		41.3		40.8		45.3		41.7		43.1	
p		1.696		1.695		—		*		1.704		1.701		1.703		1.702		1.704		*		—	
%G+C from p		36.4		35.9		—		*		44.8		41.9		43.4		42.6		44.9		*		—	

*Total DNA preparations were purified from tissue homogenates (1 g per 5 ml 0.15 M NaCl, 0.1 M EDTA; pH 8.0) by treatment with SDS and Pronase¹⁵, followed by two cycles of banding at equilibrium in neutral CsCl buoyant density gradients¹⁶. Density satellite DNAs were not detected in these preparative gradients and each DNA appeared as a single band in analytical neutral density gradients. Doublet frequencies of the purified DNAs were determined as described by Josse *et al.*⁹ with minor modifications^{17,18}. The percentage base compositions and G+C contents were determined from doublet frequency data. Also given are the G+C contents derived from the buoyant density measurements shown. Exceptions are *Antedon** and coelacanth* where the molecular weights of the isolated DNAs were too low for accurate evaluation of the peaks. Doublet frequencies are expressed in doublets per 1,000 and doublets shown in pairs are the dependent doublets^{7,8}, that is, those doublets which are complementary in the anti-parallel strands and are theoretically present with equal frequencies in double-stranded DNA. The observed values therefore give no measure of the frequencies on any one strand of the DNA. Doublets shown singly are the independent doublets, each being its own anti-parallel complement with the observed values being true for either strand of the DNA.

It can therefore be concluded that, in terms of gross sequence characteristics, protochordate DNA more closely resembles the DNAs of the invertebrates, echinodermata and brachiopoda, than the DNAs of the craniates. Other invertebrate DNAs which have so far been examined (for example, arthropod DNAs; G.J.R. and J.H.S.-S., in preparation) are even more dissimilar to the craniate pattern than the protochordate, echinoderm and brachiopod DNAs.

On the basis of taxonomic criteria^{1,2}, amphioxus has become accepted as the 'prototype' chordate closely related

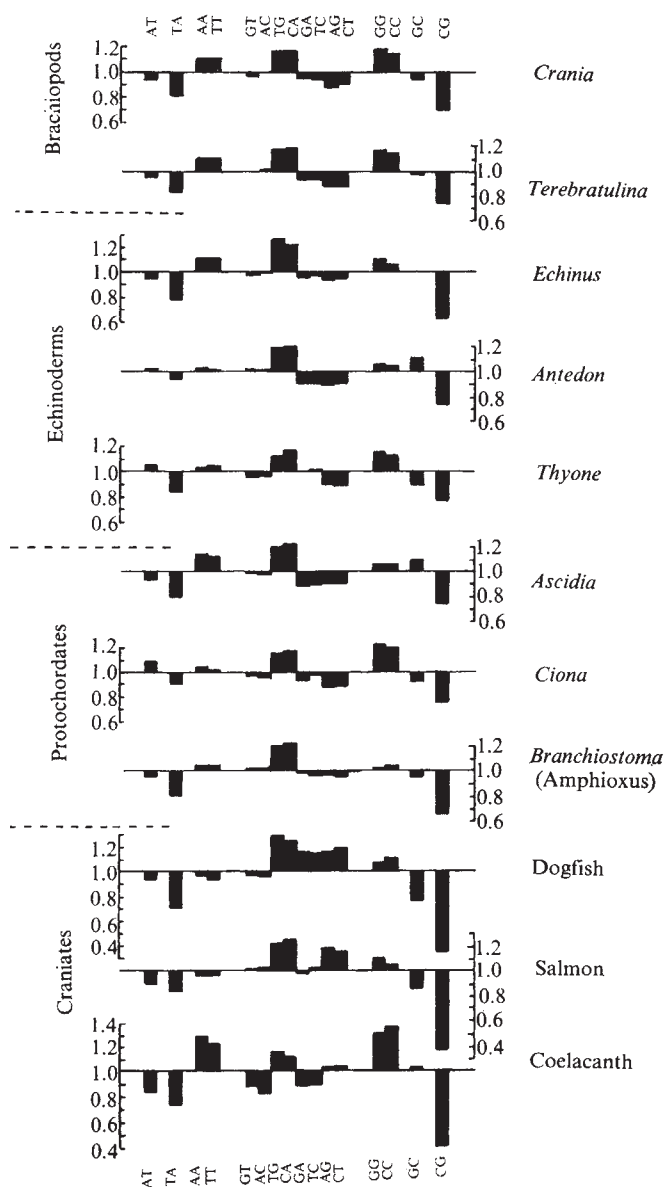


Fig. 1 Deviations from random expectation. Patterns of deviation from random expectation were determined from the doublet frequencies and base compositions shown in Table 1 by calculating the ratios of the observed doublet frequencies to the expected random frequencies⁸. The expected random frequency of a particular doublet is the product of the frequencies of the bases comprising that doublet. Both doublet frequencies and the base frequencies were expressed as fractions of unity in order to determine the ratios. A doublet with a random frequency would therefore give a ratio of one and the results are expressed in histogram form as deviations from unity. The doublet CG in salmon, for example, has a frequency of 17 doublets per 1,000 (0.017). The two bases, C and G, occur with frequencies of 22.1% (0.221) and 21.0% (0.220) respectively. The expected random frequency of a two-base sequence containing C and G is therefore $0.221 \times 0.220 = 0.046$, and the ratio of the observed frequency of CG to the expected random frequency is therefore $0.017/0.046 = 0.37$, that is, salmon DNA has only 0.37 times the expected amount of CG.

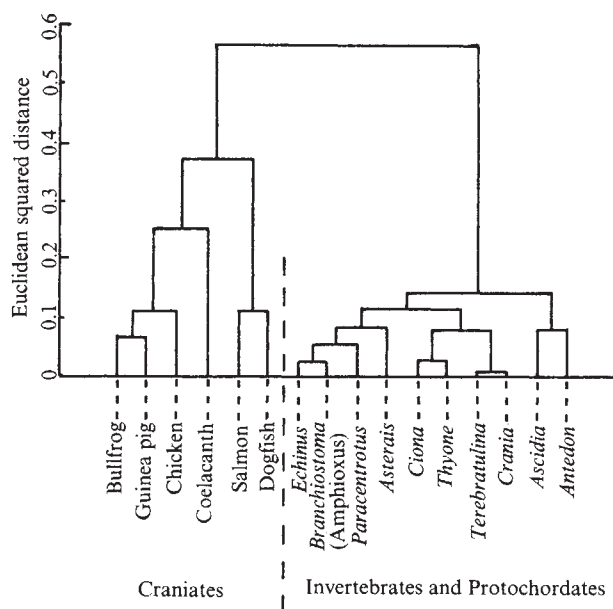


Fig. 2 Cluster analysis of the general designs of vertebrate, protochordate, echinoderm and brachiopod DNAs. The weighted average linkage method was used with Euclidean squared distance (that is, the sum of the squares of differences of ratios of observed to random frequencies between two DNAs) as the measure of distance¹⁴. The general design values for chicken, salmon, *Echinus*, *Paracentrotus* and *Asterias* DNAs were calculated from the results of Swartz *et al.*¹⁰. The values for bullfrog DNA are from McGeoch¹⁹ and those for guinea pig DNA are from Russell *et al.*¹³.

to the ancestors of the earliest craniates^{3,4}. However, using the criterion of enzyme polymorphism, Manwell has now suggested that the protochordate amphioxus is much more like invertebrates than vertebrates. Our findings, based on a very stable general characteristic of the total genome, not only provide strong support for Manwell's observation, but also show that the protochordates as a group relate much more closely to the higher invertebrates than to the craniates.

Diversification of general designs in other invertebrate phyla are being further investigated for comparison with existing phylogeny. There clearly remains a wide gap between the protochordates (acrania) and craniates, and analyses of other primitive chordates, such as cyclostomata (lamprey), are obviously needed.

The doublet frequencies for *Echinus esculenta* and salmon are from Swartz *et al.*¹⁰. We thank Dr R. H. Harris, Liaison Officer of the Coelacanth Committee of the Royal Society, for providing the coelacanth tissue.

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Haemoglobin crystals in the midgut of the tick *Ornithodoros moubata* Murray

Ticks of the *Ornithodoros moubata* Murray (Ixodoidea, Argasidae) complex, distributed through East Africa, are known as vectors of African relapsing fever¹. A laboratory strain of *O. moubata* *porcinus* (originally from Tanzania and bred according to the method of Geigy and Herbig²) uses the guinea-pig (*Cavia porcellus* L.) as experimental host. Guinea pig blood crystallises more easily than that of other hosts² in the gut of blood-sucking arthropods³. In *O. moubata* crystals appear 5–10 d after the blood meal, first small (0.015–0.05 mm) and clear red, later up to 0.2 mm in all three directions and dark red. These 'tick-grown' crystals may still be present after even more than a year. They possibly serve as nutrient reserve⁴ since they finally disappear after prolonged starvation. We describe here a study of the formation and structure of these crystals and the changes within these crystals with time. We show the crystals to be composed principally of guinea-pig haemoglobin.

O. moubata *porcinus* ticks (males and females) were taken from the laboratory strain, fed on guinea-pigs and dissected at intervals after the blood meal (0, 2, 4, 6, 12, 15, 30, 85 and 200 d), in 0.7% NaCl solution (for X-ray diffraction and spectroscopy) or without a dissecting fluid (for determination of pH and osmolality of the gut fluid).

In light microscopy of histological sections the crystals appeared as irregularly shaped triangles, with spherical haematin aggregates visible between them as dark spots. Figure 1 shows a scanning electron micrograph of the crystals. They were tentatively identified as crystals of guinea pig haemoglobin since their morphology is the same

Fig. 1 Scanning electron micrograph of haemoglobin crystals 'in' the tick midgut. Preparation: fixation 2.5% glutaraldehyde, 2% osmium tetroxide; dehydration to 100% ethanol subsequently to 100% amyl acetate, air dried, covered with carbon/gold.

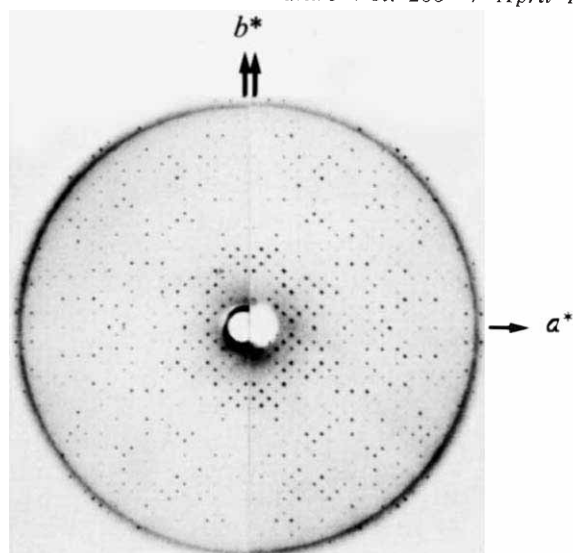


Fig. 2 Comparison of X-ray diffraction patterns of 'tick-grown' (left half) and 'in vitro-grown' (right half) crystals. 16° Precession photographs (2.8 Å resolution) of the *hk0*-zone, CuK α radiation, graphite monochromator. Arrows indicate reciprocal axes.

as described for those crystals⁵. After soaking in a standard 3 M (NH₄)₂SO₄ solution buffered with 0.1 M potassium phosphate at pH 6.7 (pH measured after 10-fold dilution) the crystals were further characterised by taking 16° precession photographs of single crystals.

Haemoglobin from guinea pigs of the same strain was purified on DEAE-Sephadex⁶. Single crystals of the major haemoglobin component (~80% of total haemoglobin) were grown from 1 and 4% solutions, using the Zeppezauer technique⁷, from either 3 M (NH₄)₂SO₄ solutions, buffered with 0.1 M potassium phosphate at pH 6–7, or from 3 M potassium phosphate solutions in the same pH range. These 'in vitro-grown' crystals were soaked in the same standard solution as the 'tick-grown' crystals and 16° precession photographs were taken for comparison.

The 'in vitro-' and 'tick-grown' crystals are both orthorhombic and, since extinctions are observed for *h+k* odd for general *hkl*- and for *l* odd for 00*l*-reflections, belong to space group C222₁. The cell dimensions are *a*=84.5±0.05 Å, *b*=89.9±0.1 Å (ref. 5), *c*=83.1±0.05 Å and they are the same for the two types of crystals. On the basis of the observed range of *V_M* values for a variety of protein crystals⁸, there can only be four haemoglobin molecules (molecular weight 64,500 each) per unit cell. The *V_M* value⁸ is then calculated to be 2.45 Å³ per dalton. The alternative of eight tetramers per unit cell would lead to the unrealistically low *V_M* value of 1.22 Å³ per dalton (<0% crystal solvent). Since space group C222₁ has eight general positions⁹ the haemoglobin tetramers must lie on the crystallographic axes parallel to either the *a* or the *b* axis as has been found for reduced horse haemoglobin¹⁰. The intensity patterns to 2.8 Å resolution are identical on visual inspection for 'in vitro-' and 'tick-grown' crystals (Fig. 2) and we therefore conclude that the 'tick-grown' crystals are in fact composed of guinea pig haemoglobin.

In order to exclude even small changes, such as scission of the chain at one or a few discrete points or limited degradation from the termini, which might explain why guinea-pig haemoglobin crystallises so readily in the midgut of *O. moubata*, we analysed the 'tick-grown' crystalline material by sodium dodecyl sulphate-polyacrylamide gel electrophoresis¹¹ (12.5% gels, cross linked 1:15). The sharpness of the protein bands was dramatically increased by using sodium phosphate instead of Tris-HCl buffer. 'Tick-grown' crystals were thoroughly washed with standard 3 M (NH₄)₂SO₄ solution, to remove any non-crystalline material,

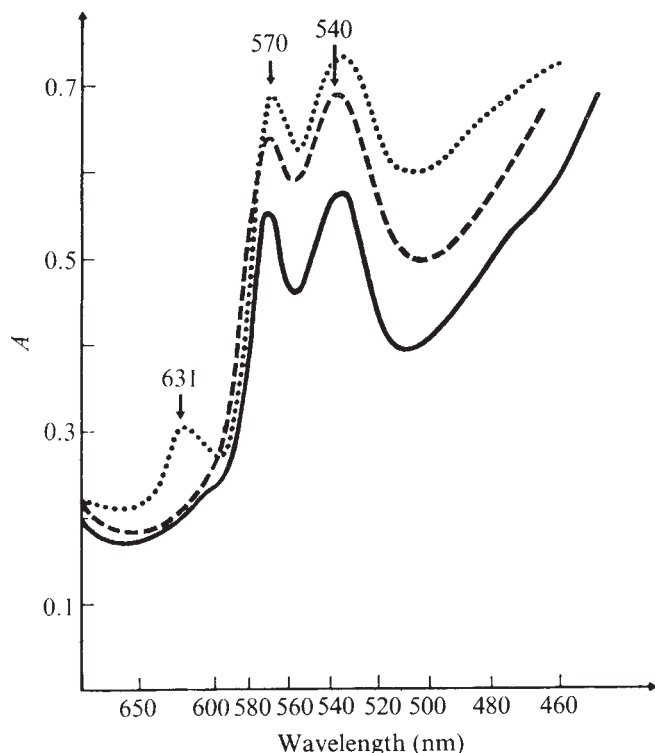


Fig. 3 Spectra from single 'tick-grown' crystals. Crystal from gut of male tick 6 d after blood meal on guinea pig (—). Crystal from gut of male tick 30 d after meal (···). Same crystal (30 d after meal) after addition of KCN to 0.01 M (---). The methaemoglobin peak at 630 nm disappeared after addition of KCN. Spectra were taken on the universal microspectrophotometer of the Wissenschaftlicher Dienst/Kriminalpolizei Zuerich. The wavelength scale is given by the dispersion of the quartz monochromator (M4QIII). Solvent was 3 M ammonium sulphate, 0.1 M potassium phosphate buffered at pH 6.7, saturated with CO. Specific absorption maxima occur for CO-haemoglobin at 540 and 570 nm, for aquomethaemoglobin at 631 nm, and for cyanmethaemoglobin at 540 nm. Conclusion—guinea pig haemoglobin crystallises in tick gut in oxy-form but transforms *in vivo* into methaemoglobin crystals with time.

and subsequently dissolved in sodium phosphate buffer. The main haemoglobin component of guinea pig blood served as a reference. In these conditions are α and β chains of guinea pig haemoglobin are clearly separated in the crystalline material and the reference haemoglobin. The R_F values are 0.452 and 0.502 for the two bands of the crystalline material and 0.448 and 0.491 for the reference haemoglobin. Calibration with lysozyme, human globin A and myoglobin and the difference in relative mobility between α and β chains of guinea pig haemoglobin yielded a difference in molecular weight of about 650. In human haemoglobin¹² the difference between α and β chains is 741 daltons. On ageing the guinea pig haemoglobin preparation gave broader electrophoretic bands as observed for carp haemoglobin¹³. The combined probe of 'tick-grown' crystalline material and fresh haemoglobin showed only two bands demonstrating the lack of significant fragmentation or extensive degradation from the termini. Removal of only one or two residues cannot be entirely excluded.

A still more sensitive test for the conformation of haemoglobin and the oxidation state of its iron in 'tick-grown' crystals is visible absorption spectroscopy on single crystals and comparison with absorption spectra of haemoglobin in solution¹⁴. Conversions of crystalline haemoglobin were followed by recording spectra from single crystals of different age (Fig. 3) obtained at different time intervals after the blood meal. Immediately after dissections the contents of the midguts were brought under a CO atmosphere to freeze the momentary equilibrium between Fe^{2+}

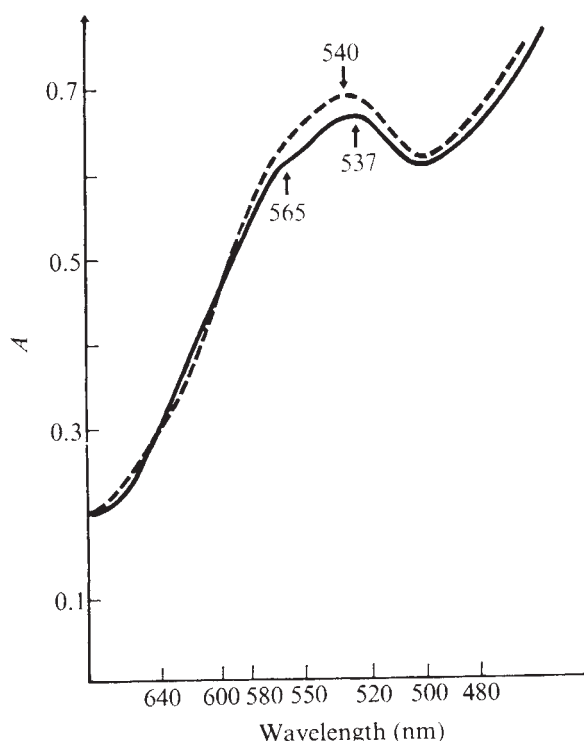
and Fe^{3+} . From Fig. 3 we conclude that haemoglobin crystallised in the tick midgut in the oxy-form but with time it transformed into methaemoglobin. This, however, is not the end state of the crystalline haemoglobin since the absorption spectra of single crystals from a tick dissected 15 months after its last blood meal show a typical haemichrome absorption spectrum with a single absorption maximum at 537 nm and a shoulder at 565 nm (Fig. 4, solid line). On soaking in a cyanide-containing solution (standard 3 M $(\text{NH}_4)_2\text{SO}_4$, 0.01 M KCN) the absorption maximum at 537 nm shifts to 540 nm and the shoulder at 565 nm almost disappears (Fig. 4, dashed line). Thus the haemichrome is not yet entirely in its irreversible state since, on KCN addition, it can be partially converted to cyanmethaemoglobin. Apparently with time methaemoglobin in the 'tick-grown' crystals transforms *in vivo* into haemichrome. The transformation to haemichrome is not associated with cracking of the crystals since, in X-ray diffraction experiments, the haemichrome-containing crystals still diffract well and behave as single crystals.

From the above it is clear that partial degradation or conformational changes are not the cause of the crystallisation of guinea pig haemoglobin in the midgut of *O. moubata*. Other factors which could effect crystallisation are changes in pH, osmolarity, or concentration of the blood meal.

The osmolarity of midgut fluid and of guinea-pig blood determined with a Knauer Halbmikro-osmometer was 300 milli-equivalent per kg. Changes in the osmolarity are therefore not responsible for the crystallisation.

Directly after a meal the midgut fluid pH is 7.5. It drops later to 6.5. This decrease initiates earlier in males (from the 6th day after the meal) than in females (from the 12th day). It is not clear whether this pH drop triggers the crystallisation. Other rodent haemoglobins are relatively insoluble in acid environment¹⁵. The decrease in pH could

Fig. 4 Spectra from single 'tick-grown' crystal. Crystal showing spectrum of haemichrome (—). Same crystal, spectrum partially converted to cyanmethaemoglobin after addition of KCN to 0.01 M (---). Conditions as in Fig. 3. Conclusion—methaemoglobin crystals *in vivo* undergo transformation into haemichrome crystals with time. This is not associated with cracking of the crystals.



also be caused by loss of haemoglobin from solution on crystallisation. *In vitro* experiments show that a decrease in pH results in an increased solubility of guinea pig haemoglobin. So it seems more likely that the decrease in pH is a result of the crystallisation (and of cellular disintegration processes) rather than its cause.

Concentration of the meal takes place by elimination of water through the coxal organ¹⁶. The concentration factor of guinea pig haemoglobin in the midgut fluid compared with haemoglobin in guinea pig blood was determined by Drabkin's method¹⁴ on 10 μ l of fluid or blood. For ticks, dissected 2 h 45 min after their blood meal, a concentration factor of about 1.9 was found which compares well with the value of 1.72 found by Kaufman¹⁷ 2 h after completion of the blood meal. Crystallisation in the midgut starts only after several days, thus the initial, about 2-fold, concentration of the meal is not directly responsible for it. It is, however, possible that concentration of the meal, after the initial steep phase, goes on at a reduced rate and thus eventually triggers crystallisation.

Guinea pig haemoglobin crystallises in the midgut of the tick *O. moubata* as oxyhaemoglobin and, with time, transforms to methaemoglobin and then to haemichrome without disruption of the crystals. Partial haemoglobin degradation or changes in pH or osmolarity of the midgut fluid are not responsible for crystallisation. Continued concentration of the blood meal may have a role.

In tick biology, the crystals seem to have an important but not essential role in the preservation of the nutrient reserves since Argasids fed with human blood (which does not crystallise²) may also starve for very prolonged periods. It is not known how the nutrient stored in the crystal is made available for intracellular digestion by the starving tick. Distortion of the equilibrium between solid state (crystals) and liquid state (midgut fluid) by the digestive activity of the midgut cells, however, could lead to a gradual solvation of the crystals.

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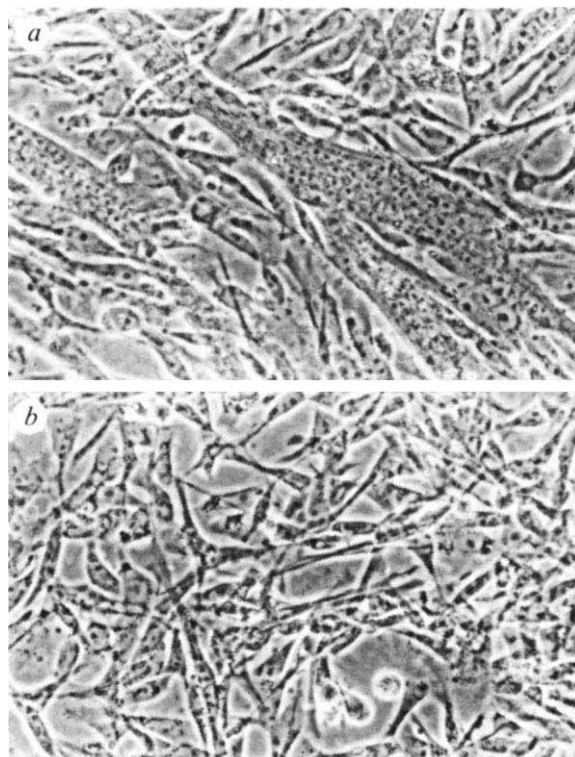
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Effect of a tumour promoter on myogenesis

EVIDENCE that viruses and chemical carcinogens alter the ongoing synthetic programme of cells has been presented^{1,2}. Chondroblasts and melanoblasts transformed with a temperature-sensitive Rous sarcoma virus cease synthesising the chondroblast-unique sulphated proteoglycan and melanin, respectively³. Shifted to non-permissive temperature, these cells re-initiate the synthesis of the chondroblast-unique sulphated proteoglycan and melanin. Similar results have been obtained using chick myogenic cells⁴⁻⁵. Normal presumptive myoblasts undergo a limited number of replications, yielding postmitotic, mononucleated myoblasts. Postmitotic myoblasts initiate the synthesis of muscle-specific myosin heavy and light chains and fuse into myotubes^{6,7}. In contrast, ts-viral transformed myogenic cells continue to replicate, do not initiate the synthesis of muscle-specific myosins and do not fuse. 24-48 h after shifting to non-permissive temperature, many transformed myogenic cells withdraw irreversibly from the cell cycle, initiate the synthesis of muscle-specific (unpublished data) myosins and fuse. Apparently when the transformation factor is expressed the cells do not withdraw from the cell cycle, and as a consequence of being forced to cycle, do not terminally differentiate. To probe the mechanisms

Fig. 1 Phase micrographs of living 4-d primary muscle cultures grown in *a*, normal medium and *b*, normal medium plus PMA. The myotubes may achieve lengths of over 1 mm, may contain hundreds of postmitotic nuclei, and contract spontaneously. The replicating mononucleated cells in both cultures are in various compartments of the myogenic and fibrogenic lineages, and cytologically the cells in these lineages cannot be distinguished. The vast majority of cells in PMA-treated cultures are small bipolar cells. Under higher magnification occasional bi- and tri-nucleated cells are observed in the PMA-treated cultures; the frequency of such cells is less than 1 per 10³ mononucleated cells. The multilayered arrangement and overlapping of cell processes demonstrates the absence of density-inhibited replication and migration in the PMA-treated cultures.



that might be common to the generation of terminally differentiated cells from normal precursor cells, and to the generation of malignant cells from normal precursor cells, we studied the effects of phorbol-12-myristate-13-acetate (PMA) on myogenesis. Of the purified phorbol esters, PMA is the most potent as a tumour-promoting agent and as a mitogen⁸⁻¹². We report here that PMA mimics some of the effects of the Rous sarcoma tumour agent on myogenesis.

Primary myogenic cultures were prepared from muscles of 11-d-old chick embryos¹³. PMA (5×10^{-8} M) was added at various times. Muscle-specific myosin heavy chains were assayed, using antibodies specific for muscle heavy chains. Some properties of these myosin antibodies are detailed in Fig. 2 and refs 6 and 7. The actomyosin was extracted from cells by the method of Adelstein *et al.*¹⁴. Muscle-specific myosin light chains were determined by running partially purified actomyosin extracts in SDS-polyacrylamide gels^{7,15}. Postmitotic, mononucleated myoblasts were collected by treating primary cultures with Cytochalasin-B^{6,16} or EGTA plus cytosine arabinoside (Ara-C)^{7,17}.

A micrograph of a control 4-d culture is shown in Fig. 1a. Over 50% of all nuclei were in myotubes. The myosin heavy chains from these cultures formed a single precipitin band with antisera against muscle myosin (Fig. 2). Actomyosin extracts in SDS-polyacrylamide gels exhibited muscle-specific, fast myosin light chains, LC₁ and LC₂, with molecular weights of 25,000 and 18,000 (data not shown)^{7,15}.

Figure 1b is a micrograph of a culture reared continuously in PMA for 4 d and clearly shows the absence of myotubes. Myosins from these cells did not form precipitin bands with antibody against muscle-specific myosin (Fig. 2). Furthermore, the light chains from these cells co-migrated with the light chains from presumptive myoblasts or fibroblasts and have MWs of 20,000 and 16,000.

When myogenic cells grown in PMA for 6 d were shifted to normal medium, many withdrew from the cell cycle and terminally differentiated within 24–48 h. In another series cells were subcultured in PMA for 1 or 2 passages. During each passage the number of cells increased over sevenfold (Table 1). The cells did not fuse during these subcultures. At the end of the first or second subculture the cells were shifted to normal medium. Within 24–48 h many cells withdrew from the cell cycle, fused and terminally differentiated. The ratio of myotube nuclei/total nuclei in these 'reversed' cultures equalled that of primary cultures. These findings, and additional

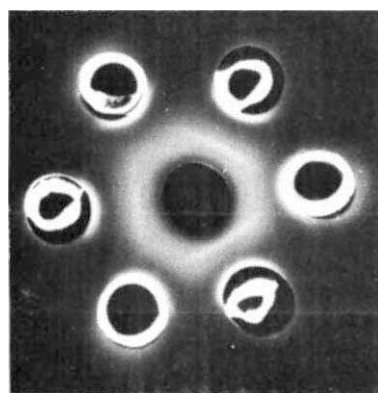


Fig. 2 Control and PMA-treated cultures were extracted for actomyosin and diffused against antibody to adult chicken skeletal myosin. In the experiment shown, this particular myosin antibody in Ouchterlony tests did not form precipitin bands against C protein²⁸. Similar antibodies did not precipitate myosin from chicken cells (replicating presumptive myoblasts, fibroblasts, mesenchyme, smooth muscle, embryonic nerve cells or Rous-

transformed myogenic cells grown at permissive temperature) or human blood platelets or mouse L cells. This antibody did form precipitin bands with actomyosin extracted from (1) postmitotic, mononucleated myoblasts, (2) normal myotubes and (3) myotubes that formed when Rous sarcoma ts-transformed myogenic cells were reared at non-permissive temperature (refs. 6, 7, 16, 18 and unpublished results). Actomyosin solutions, antibody solutions and gels were equilibrated with 0.4 M KCl 0.03 M phosphate buffer, pH 7.3: 40 μ l of solution were placed in each well and allowed to diffuse at 4 °C for 48 h. The gels were washed on phosphate buffer for 24 h, stained with thiazine in H₂O and destained with 1% acetic acid. Wells are numbered clockwise and the upper right-hand well is no. 1. The centre well contains antibody to adult skeletal chick myosin. Wells 1, 3 and 5 contain 4 mg ml⁻¹ actomyosin from control cultures. Wells 2, 4 and 6 contain 5 mg ml⁻¹ actomyosin from PMA-treated primary cultures. In other experiments, similar results were obtained when the protein concentration of actomyosin from cells reared in PMA was four times that of control cultures. Lastly, fluorescein-labelled antimyosin experiments performed as described in refs 7, 25 yielded results consistent with the agar diffusion tests. For this photograph the stained gel served as a negative.

autoradiographic data are shown in Table 1. The mitogenic effect of PMA (Table 2) preceded fusion, which in these cultures begins on day 3 and peaks on day 4. This type of mitogenic effect with PMA is also observed with ts-transformed myogenic cells and with cells that have incorporated BudR into their DNA¹³.

The following experiments demonstrate that the mitogenic target of PMA was the replicating presumptive myoblasts and fibroblasts, and that PMA was not a mitogen for nuclei in the definitive, mononucleated myoblasts or the nuclei within myotubes. Mononucleated myoblasts synthesising muscle-specific myosins were collected by treating primary cultures with cytochalasin-B or EGTA

Table 1 Autoradiographic analysis of standard, PMA-treated and PMA-reversed myogenic cultures

	% of all labelled nuclei per dish		% of all unlabelled nuclei per dish	
	Nuclei in mononucleated cells	Postmitotic nuclei in myotubes	Nuclei in mononucleated cells	Postmitotic nuclei in myotubes
Standard 4-d primary cultures	26	0.2*	28	46
4-d PMA-treated primary culture	20	<0.1*	25	55
PMA-treated primary culture	51	0	49	0
PMA-treated primary culture subcultured into normal medium	46	0	52	0
PMA-treated primary culture subcultured into PMA-medium	28	1*	20	51
PMA-treated primary culture subcultured into PMA-medium	52	0	48	0
PMA-treated primary culture subcultured into PMA-medium	59	0	41	0

Late 3-d cultures were exposed to ³H-TdR for 10 h, processed for autoradiography, and cells counted as described¹³. Initial inoculum 0.5×10^6 nuclei per 35-mm Petri dish. In standard 4-d cultures there were 3×10^6 to 4×10^6 nuclei per dish, of which roughly 50% were postmitotic and in myotubes. In PMA-treated primaries there were 4×10^6 to 5×10^6 nuclei per dish, virtually all mononucleated. Secondary cultures of PMA-treated cells if again grown in PMA achieved densities of up to 6×10^6 nuclei per dish after 4 d. If, however, PMA-treated primaries were grown as secondaries in normal medium, they again achieved densities of 3×10^6 to 4×10^6 nuclei per dish, of which 50% were postmitotic and in myotubes. Additional experiments are required to determine what percentage of unlabelled mononucleated cells in both types of culture were in fact (1) definitive postmitotic myoblasts, and (2) myogenic and fibrogenic cells that simply did not enter S during the period in ³H-TdR. It should be stressed that normal and PMA-treated cultures did not cease replicating when confluent, but multilayered.

*The small percentage of labelled nuclei in myotubes were due to the fusion of labelled daughter cells whose mothers passed through at least the later part of S and then divided during the 10 h the cells were in ³H-TdR.

Table 2 DNA determination of controls and PMA-treated cultures

Type of culture	DNA content (μg per dish) at various times			
	48 h	72 h	96 h	120 h
Control primary	52	93	144	144
PMA-treated primary	70	147	235	292

Each value (mean of two separate experiments performed at different times) represents the amount of DNA (in μg) per 100 mm Petri dishes. Initial inoculum 0.5×10^6 cells per ml, 8 ml per dish. DNA was determined by the method of Burton²⁴. The values for the 120-h cultures are difficult to interpret. In both types of cultures cells are lost into the medium. In the controls, entire multinucleated myotubes often degenerate, whereas in the PMA-treated cultures many mononucleated cells are displaced from the substrate and die.

plus Ara-C^{6,7,18}. These definitive myoblasts were subcultured into medium with PMA and ³H-TdR or with ³H-TdR alone, and both series autoradiographed. Less than 2% of the cells incorporated the label after 36 h in both series. In subcultures prepared from untreated primaries, over 70% of the cells were labelled after 36 h. Not a single nucleus within a myotube was labelled when PMA plus ³H-TdR was added after myotubes had formed.

Fusion is the end step of several distinct events. First, the generation by way of cell divisions of postmitotic, fusible-competent myoblasts¹⁸. Second, the mutual recognition, lateral alignment and adhesion of these postmitotic myoblasts¹⁹⁻²¹. And third, the physical event of fusion itself. Fusion, as determined with living cells under the phase microscope, requires no more than 30-60 min^{22,23}. The following demonstrates that, in addition to acting as a mitogen, PMA blocks the physical event of fusion. Fusible-competent, postmitotic myoblasts were collected from cultures treated with cytochalasin-B or EGTA plus Ara-C. When these myoblasts were subcultured into normal medium, over 60% fused within 15-20 h. When these definitive myoblasts were subcultured into medium with PMA, fusion was blocked, although as mononucleated cells they continued to synthesise the muscle-specific myosin heavy and light chains.

With respect to promoting cell replication, PMA-treated myogenic cells mimic the behaviour of myogenic cells transformed with ts-Rous sarcoma virus, and of myogenic cells that incorporated BudR into their DNA. Possibly PMA, the ts-virus and BudR all exert their common effect on myogenesis by forcing cells in the penultimate compartment of the myogenic lineage to replicate. In addition, however, PMA, but not the virus or BudR, blocks the event of fusion. It may be that PMA acts directly on the cell surface of replicating presumptive myoblasts and postmitotic myoblasts. The former are inhibited from pulling out of the cell cycle, the latter from fusing. In any case, the final effect of such dissimilar agents as Rous virus transforming factor, BudR, and PMA seems to be to force presumptive myoblasts to undergo proliferative cell cycles which in turn precludes the flipping of the 'master-switch' which permits activation of the synthetic program of the post-mitotic mononucleated myoblast^{5,7,20,25}.

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Human LD antigens are present on xenogeneic cells

THE mixed leukocyte culture (MLC) reaction is largely a measure of specific responsiveness of thymus-derived lymphocytes to foreign allogeneic and xenogeneic major histocompatibility complex (MHC) controlled LD antigens¹. In view of the remarkable primary structure homologies of MHC-controlled SD antigens between humans and mice², we have investigated whether MHC LD antigens have also been conserved in evolution by studying the extent of LD sharing between different species. Using the primed lymphocyte typing (PLT) test, we have found extensive sharing of LD antigens between humans and monkeys, and less, but still significant, sharing between humans and dogs and humans and cattle. In no case was there evidence that mouse LD antigens were shared with those of humans.

Human lymphocytes cultured in primary allogeneic MLC proliferate extensively and revert to small lymphocytes by day 10. If then restimulated with the original sensitising cell these primed lymphocytes (PLT cells) give a much more rapid proliferative response, which can be assayed by ³H-TdR incorporation as early as 24 h. This secondary, or typing, response is specific for HLA I.D determinants and is also elicited by random restimulating cells (test cells) that share LD determinants with the original sensitising cell³. PLT cells can also be generated in xenogeneic combinations and appear specific for the I.D antigens of the sensitising species⁴.

If individual human cells are sensitised to a pool of 20 allogeneic stimulating cells, the resulting PLT cells (pool-PLT cells) seem sensitised to essentially all human I.D determinants, as virtually any human test cell used for restimulation elicits a strong secondary response⁵. Test cells that elicit a poor secondary response from a pool-PLT cell must either share LD determinants with the responding cell or carry LD determinants that are not represented in the sensitising cell pool. The primary MLCs between test and responding cells distinguish between these two possibilities. The magnitude of the secondary response correlates very well with that of the primary MLC ($r=0.89$) (Fig. 1). This indicates that human test cells that fail to restimulate a pool-PLT cell fail to do so because of LD determinants shared with the responding cell. The sensitising cell pool thus seems to include essentially all human LD determinants.

Restimulation of a human pool-PLT cell with a xeno-

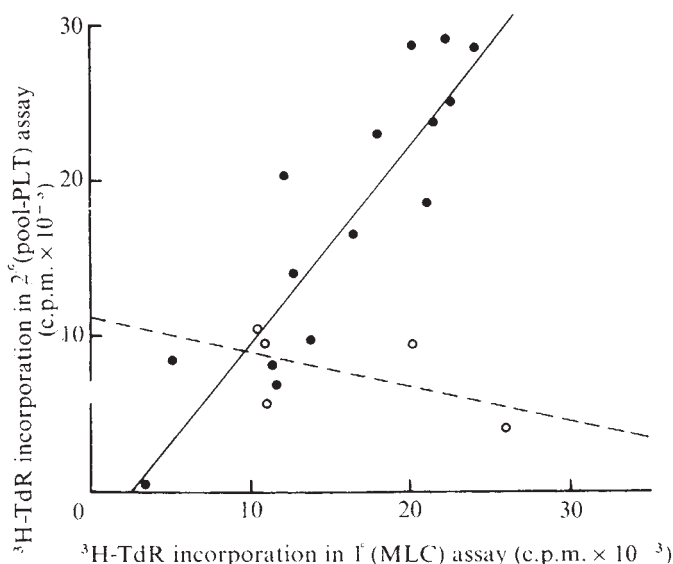


Fig. 1 Correlation of MLC and pool-PLT responses. Pool-PLT cells were generated and assayed as follows: 1×10^7 human responding cells were cultured with 1×10^7 irradiated, pooled human stimulating cells (consisting of 20 random donors) in upright flasks (Falcon No. 3013) in 30 ml RPMI 1640 medium supplemented with 25 mM HEPES buffer, penicillin, streptomycin and 10% heat-inactivated pooled human plasma. After 10 d the cells were collected, resuspended in fresh supplemented medium and distributed to flat-bottomed microtitre plates (Linbro No. IS-FB-96TC). 10^5 pool-PLT responding cells were restimulated with 2×10^5 irradiated human (●) or monkey (○) stimulating cells per well. Cultures were labelled with ^3H -TdR for 8 h on day 2 after restimulation. Primary MLCs were done using 10^5 fresh human responding cells and 2×10^5 irradiated human (●) or monkey (○) stimulating cells per well and were labelled for 8 h on day 5 of culture. —, least squares regression line for human stimulating cells ($r = 0.89$, $P < 0.01$); least squares regression line for monkey stimulating cells ($r = -0.55$, not significant).

genic test cell should give a positive response if LD determinants on the xenogeneic test cell are shared with human LD determinants. Data for four species whose cells were tested for their ability to restimulate a pool-PLT cell (Table 1) show that in four experiments with five different pool-PLT cells, cells from all 10 rhesus monkeys tested

caused significant restimulation. This restimulation was 44–135% of the average restimulation caused by a panel of random human test cells. There is a decreasing percentage of positive responses as well as percentage of restimulation by those cells that are positive. The primary MLC data show that those xenogeneic test cells that do not restimulate the pool-PLT cell still elicit a good primary MLC response from the same responding cell, indicating that the LD determinants recognised on those xenogeneic cells in a primary MLC are not represented in the human sensitising cell pool.

The data obtained with the pool-PLT test show that LD antigens are shared between species. The degree of sharing is quite extensive in rhesus monkeys, where all animals tested shared antigens with human cells, and less in dogs and cattle. In the 17 mouse strains we tested (11 independent and 6 recombinant H-2 haplotypes) there was no evidence for LD antigens shared with human cells.

Even in rhesus monkeys, where all animals so far tested have given positive secondary responses with pool-PLT cells, shared specificities do not seem to be the sole basis of recognition. In addition to the human cells shown in Fig. 1, five rhesus cells were also tested. All of these cells gave positive secondary responses, but the correlation between primary and secondary responses was poor ($r = -0.55$, not significantly different from zero), suggesting that there are additional LD determinants on the xenogeneic cells, not shared with human LD determinants, that are still recognised in a primary xenogeneic MLC.

Estimates of the frequency of initially responding cells in MLC to any one haplotype range from 1 to 6% (refs 6–8). As well as extensive polymorphism for LD antigens, in at least some cases the LD antigens controlled by two different haplotypes in the same species seem to be non-cross reactive^{4,9}. We thus have to account for the ability of an individual animal to respond to all of the different LD antigens in its species, given the apparent clonal nature of this response^{10,11}.

That lymphocytes can also respond to xenogeneic cells in MLC^{12,13}, and that this response is specific and apparently directed towards the MHC LD antigens of the sensitising species⁴, further compounds the problem in a quantitative sense. To overcome this difficulty it has been suggested that allogeneic recognition is unique¹⁴ and, to the extent

Table 1 Pool PLT-cells restimulated by allogeneic or xenogeneic test cells*†

Xenogeneic species tested	48 h‡ pool-PLT	5 or 6 d‡ 1° MLC	No. of experiments (no. of pool-PLT cells)	No. positive§ Total no. tested	Range of restimulation by positive cells (median value)
Rhesus monkey	AP _x + A _x 2,949¶ + B _x 55,521 + X _x 30,418 + Y _x 24,183	AA _x 1,005¶ AB _x 37,692 AX _x 26,811 AY _x 13,173	4(5)	10/10	44–135% (71%)
Dog	AP _x + A _x 1,943 + B _x 26,194 + X _x 11,821 + Y _x 14,263	AA _x 1,205 AB _x 31,527 AX _x 17,367 AY _x 12,973	5(6)	13/14	11–97% (36%)
Cow	AP _x + A _x 2,279 + B _x 33,485 + X _x 12,279 + Y _x 4,509	AA _x 1,173 AB _x 31,723 AX _x 5,920 AY _x 13,713	5(5)	8/30	11–35% (17%)
Mouse	AP _x + A _x 3,603 + B _x 24,835 + X _x 3,462 + Y _x 3,254	AA _x 3,299 AB _x 39,750 AX _x 19,450 AY _x 14,470	5(7)	0/34	—

*All human responding cells are designated A, although different individuals were used as responding cells in each experiment.

†A_x, B_x: irradiated human stimulating cells.

X_x, Y_x: irradiated xenogeneic stimulating cells of species indicated.

‡Cultures were performed as in Fig. 1 legend, using 2×10^5 irradiated monkey or dog stimulating cells (peripheral blood lymphocytes), 8×10^5 irradiated cattle stimulating cells (peripheral blood lymphocytes), or 10×10^5 irradiated mouse stimulating cells (spleen cells depleted of erythrocytes by hypotonic shock) per well.

§All animals scored as positive gave restimulation values significantly different from autologous controls ($P < 0.05$).

||Expressed as percentage of average restimulation by a random panel of human test restimulating cells.

¶c.p.m. ^3H -TdR incorporation.

that it occurs, xenogeneic recognition simply represents recognition of those xenogeneic antigens identical to, or cross-reactive with, the LD antigens of the responding species¹⁵. This would considerably limit the number of foreign MHC LD antigens that any animal would need to recognise. Our data indicate, however, that this is not the case. The very significant xenogeneic MLC seen between human and mouse cells, which seems to reflect specific recognition by human cells of mouse H-2 LD antigens, suggests that xenogeneic antigens can be recognised in MLC even when they are not cross-reactive with human LD antigens.

Thus we are left with the high frequency of initially responding cells in MLC, which must include specific recognition of allogeneic as well as non-cross-reactive xenogeneic LD antigens. Models that could explain this high frequency of initially responding cells have been discussed before¹⁶⁻¹⁸. One possibility is that the LD antigens coded for by different haplotypes within a species are to a large extent cross-reactive. The high frequency of responding cells in the rat system with the lack of any sharing of LD antigens in the few haplotypes tested could be the unusual case⁹. The very low number of MHC haplotypes existing in rat¹⁹ is in marked contrast to mice²⁰ and humans²¹, where cross-reactivity may be extensive. In fact, the high degree of LD sharing between humans and rhesus monkeys, and the still significant homologies with more distantly related species such as dog and cow, supports the concept that the large number of phenotypes that exist in all species may be based on the expression of different combinations of a limited number of determinants.

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Circulating immune complexes in normal human pregnancy

CIRCULATING antigen-antibody (AgAb) complexes are important pathogenic factors in various diseases. In addition to their inflammatory effect, immune complexes can interfere with cellular immunity. For instance, in cancer, the so-called blocking factors are apparently circulating AgAb complexes^{1,2}. Factors interfering with cellular immunity specifically directed against placental and foetal antigens have also been described in

pregnancy serum³⁻⁵, and some data suggest a role of immune complexes as blocking factors in pregnancy⁶. We report here that circulating AgAb complexes are a constant feature of every normal pregnancy. This, of course, raises interesting questions as to the possible involvement of such complexes in the tolerance of the foetus by the mother.

Agglutination inhibition tests were carried out as before⁷ using latex suspension provided by the Behring Institute (Marburg, FRG). However, human rheumatoid factor (RF) or Clq which failed to react with the immune complexes of pregnancy, were replaced by rabbit RF. RF was induced in rabbits by injecting 1 mg of autologous aggregated IgG mixed with complete Freund's adjuvant intradermally every 2 weeks. IgG was isolated by DEAE-cellulose chromatography and aggregated by heating at 63 °C for 10 min. For the isolation of RF, the animal sera were passed through a column of human IgG coupled to 6-aminohexyl-Sepharose 4B activated by glutaraldehyde⁸. The fraction eluted between 1 M and 2 M NH₄SCN was used after dialysis against saline. This fraction consisted almost exclusively of IgM.

Rabbit RF was inhibited by all sera from non-pregnant women ($N=14$) at dilution titres ranging from $\frac{1}{4}$ to $\frac{1}{16}$, whereas the inhibition titres of 84 sera collected from 55 women at various stages of normal pregnancy before the 38th week, ranged from $\frac{1}{32}$ to $\frac{1}{256}$ (Fig. 1). Those samples collected after the 38th week had a titre of $\frac{1}{16}$. The courses of two typical pregnancies showed an increase up to $\frac{1}{128}$ of the RF inhibition titre with the advancement of pregnancy. At 3-4 weeks before delivery the inhibition titres dropped to levels observed in non-pregnant women.

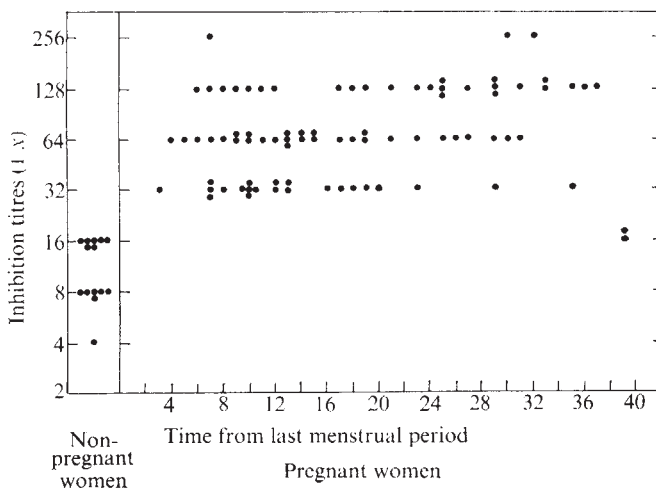
In an attempt to characterise the inhibiting factors, fresh sera from six pregnant women (gestation of 12-37 weeks) and with RF-inhibition titres ranging from $\frac{1}{64}$ to $\frac{1}{256}$ were fractionated by gel filtration (Fig. 2).

In all sera from pregnant women, a high inhibitory activity towards RF was detected in the fractions corresponding to molecular sizes $\geq 10^6$. Sera from non-pregnant women were devoid of these large size inhibitory factors but, like sera from pregnant women, contained inhibitory factors eluted in the 7S region. In general, this inhibitory activity was maximal in the ascending slope of the peak of IgG (Fig. 2).

All sera from pregnant and non-pregnant women contained, in addition to 7S IgG, a small amount of this immunoglobulin eluted in a region corresponding to a molecular size ranging from 500,000 to several millions. On the average, 'heavy' IgG corresponding to 0.27% (s.d. 0.20) of our standard was recovered from 1 ml pregnancy serum, and 0.21% (s.d. 0.16) from 1 ml of control serum. These two quantities did not differ significantly. In one pregnant woman, both IgA and IgG were detected in the elution zone of IgM.

The C3 factor of complement was also found to be dis-

Fig. 1 Inhibitory activity toward rabbit RF of sera from pregnant and non-pregnant women.



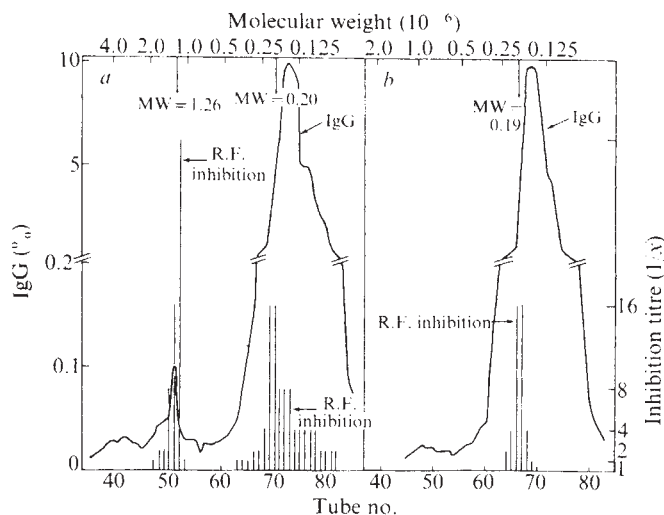


Fig. 2 Gel filtration of two serum samples (3 ml) on a calibrated Ultrogel AcA 22 column. *a*, Serum from a 24-week pregnant woman. *b*, Serum from a non-pregnant woman. The fractions of 5 ml were analysed by nephelometric immunoassay of IgG (Technicon AIP system) and tested for their inhibitory activity towards rabbit RF. The concentrations of IgG were expressed in percentage of the IgG content of a pool of normal sera. Presence of IgA, IgM and C3 complement were followed similarly. Sera from age-matched non-pregnant women served as controls.

tributed in two fractions. The major one was detected, as was expected, in the ascending slope of the IgG peak, and the minor one in the elution zone of heavy IgG. In contrast to IgG, however, a highly significant difference ($P < 0.001$) was observed between the amounts of 'heavy' C3 occurring in pregnant and non-pregnant women. The pregnancy sera contained heavy C3 corresponding to 2.5% (s.d. 0.41) of our standard and the control sera 0.46% (s.d. 0.37).

Sometimes, the peak of heavy IgG or heavy C3 did not exactly match the peak of heavy inhibitory factors. Such a discrepancy was also observed in the fractionation of sera from patients with immune complex diseases. A possible cause could be the extreme sensitivity of RF for immune complexes of a certain size⁷.

To determine whether the inhibitory factors of large molecular size corresponded to antigen-antibody complexes, the corresponding fractions were concentrated 10-fold by ultrafiltration, dialysed against 0.1 M HCl-glycine buffer, pH 3.0 to dissociate the complexes, and passed through a column of Sephadex G200 in the same buffer to separate the antigen from the antibody. Five peaks of material absorbing at 280 nm were obtained. After neutralisation by addition of 100 μ l of 1 M Tris solution to the 5-ml fractions, they were analysed for IgG content by immunonephelometry and for their inhibitory activity toward RF. All fractions were found to be devoid of inhibitory factors. IgG was detected in the third fraction which was eluted in the position expected for a 7S protein.

A sample of 200 μ l of each fraction was mixed with the same volume of each of the other fractions. After incubation at 37 °C for 30 min. and overnight at 4 °C, the 10 mixtures were titrated for their inhibitory activity. Only the mixture of the IgG-containing fraction with the second fraction gave an inhibition which was still detectable after a 1/512 dilution. This second fraction was eluted in a position suggesting a molecular weight of about 400,000. The resolution at acid pH of the inhibitory factors into five peaks was confirmed with a serum sample of another pregnant woman, and the identification of the material constituting each of these fractions is now in progress.

It is known that rabbit RF is capable of reacting with human IgG, especially when it is aggregated or involved in AgAb complexes⁹. Our gel filtration experiments clearly show that the increase of RF inhibition occurring during pregnancy is

due to the appearance of factors of molecular sizes of between 500,000 and several millions. The presence of IgG and C3 in these heavy fractions and the dissociation-reassociation experiments leave little doubt about the AgAb complex nature of the heavy inhibitory factors. It remains to be explained, however, why rabbit RF was inhibited by heavy serum fractions from pregnant women and not from non-pregnant women, despite the fact that these fractions contained the same amount of IgG. This discrepancy might be due to a difference in the IgG subclass involved in the complexes. The hypothesis that rabbit RF would preferentially react with certain human subclasses of IgG is suggested by the elution pattern of the inhibitory factors detected in the 7S region. In sera from most pregnant and non-pregnant women, the maximal inhibition in the 7S region was found where material with a molecular size of about 170,000–200,000 was eluted. This inhibitory factor, therefore might correspond to IgG3, which has a molecular weight of 172,000 (ref. 10).

It has been reported that healthy individuals may possess IgG endowed with RF activity which can form an immune complex with other IgG molecules by a self-association mechanism¹¹. The heavy IgG that we have detected in control sera presumably correspond to this type of complex. But, the type occurring during pregnancy seems clearly different since it contains larger amounts of C3 and an antigen which was not IgG but a substance with an estimated molecular size of about 400,000. We presume that this antigen originates from placental or foetal tissues.

The detection of circulating AgAb complexes in the serum of pregnant women opens several new perspectives. In gynaecology, the determination of immune complexes seems to be a possible test for monitoring the evolution of pregnancy. In pathology these immune complexes might be recognised as the pathogenic factors of preeclampsia, especially in view of the observation that deposits of IgG and C3 have been detected in the renal glomeruli of normal pregnant guinea pigs and mice¹². In immunology, investigations of the blocking effect of immune complexes of pregnancy, and identification of the antigen and antibody involved may clarify the intriguing mechanism responsible for the immune tolerance of the mother toward the foetus.

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Y-antigen killing by T cells of women is restricted by HLA

CYTOTOXIC T cells are important in graft rejection and in the control of virus infections, but the mode of interaction of these cells with their targets, particularly in man remains unclear. It has been shown in mice that products of genes in the major histocompatibility complex (H-2) are involved in these interactions even when the cytotoxic T cells are specific for viral or non-H-2 antigens. This involvement is seen as the requirements that the target cell must express the specific non-H-2 antigen and in addition the same H-2, D or K region antigens as were present on the cells which initiated the immune response¹⁻⁴. Cytotoxic T cells specific for viral or non-H-2 antigens are thus restricted in the targets that they can kill by the H-2 antigens on the targets. For example, cytotoxic T cells from H-2^b female mice suitably primed to the Y antigen of H-2^b males, will only kill cells from male mice carrying an H-2D region derived from H-2^b (refs 5, 6). This report is to our knowledge the first clear demonstration that a similar restriction occurs in man. We describe a situation in man where cytotoxic reactions specific for non-HLA antigens can only occur when target cells carry both the HLA-A2 antigen of the original sensitising cell and the non-HLA target determinant(s). The strong association with maleness suggests that one of the specific antigens involved was coded for by the Y chromosome.

Table 1 Killing pattern of cells from patient Reef, against her own target cells and those from other family members

Targets	HLA compatibility with patient	% Killing of CML	
Donor ♂ ac*	yes	63†	18‡
Patient ♀ ac	yes	—	NT
Sib 5 ♂ ac	yes	63	NT
Sib 3 ♀ ad	no	53	13
Sib 4 ♀ ad	no	31	NT
Mother ♀ cd	no	4	0

Table 1 shows percentage of chromium release of tests in which the patient's cells were tested against members of her family. NT, not tested.

*HLA haplotypes: a, A2, BW40, W6, CW3; b, A2, BW40, W6, CW3; c, A2, B12, W4, —; d, AW31, BW35, W6, CW4.

†Results after *in vitro* sensitisation to donor.

‡Results without previous *in vitro* sensitisation. MLRs between donor, patient and sib 5 were sufficiently low to exclude the possibility of a recombination between HLA-B and HLA-D.

Our study began with an observation that a female patient (Reef) who had been suffering from aplastic anaemia rejected a bone marrow graft from her HLA-identical brother. Before this rejection, a transient chimaerism of peripheral blood cells indicated that the graft had initially taken. Rejection was accompanied by a spontaneous recovery of the patient's bone marrow haemopoietic function, thus allowing further investigations. Peripheral blood lymphocytes from the patient were used as effector cells to measure the level of cytotoxicity against target cells from the patient's family and an unrelated panel. The patient's lymphocytes were first restimulated *in vitro* by incubating them for 6 d with irradiated stimulator cells from her HLA identical brother. Cytotoxicity was measured using an isotope release assay⁷ with target cells which had been incubated with phytohaemagglutinin for 3 d and then labelled with sodium ⁵¹chromate. Effector cells (70×10^4) and target cells (1×10^5) in RPM-1640 plus 20% heat-inactivated human AB serum were incubated for 4 h in round-bottomed microtitre plates. Plates were centrifuged for 5 min at 200g and the supernatant removed and counted in a γ -counter. Each cell combination was tested in triplicate. After subtraction of background

the percentage of isotope release was calculated according to the following formula:

$$\frac{\text{experimental mean} - \text{mean of spontaneous release}}{\text{freeze-thaw mean (100\% release)} - \text{mean of spontaneous release}} \times 100 = \% \text{ kill}$$

In our initial studies performed 31 weeks after grafting, cytotoxicity was demonstrated against the donor's lymphocytes even when the patient's cells had not been restimulated *in vitro* (Table 1). This suggested that killing was to a non-HLA target as the donor was HLA identical to his sister. Three further experiments were performed using effector cells from the recipient sensitised to the donor and using cells from an unrelated panel as targets. HLA phenotypes are shown in Table 2.

Table 2 Positive and negative killing by cells from patient Reef against target cells from unrelated individuals

	HLA Phenotypes				Sex	% Kill			
	HLA-A		HLA-B				HLA-C		
(Expt 1):	1	2*	W31	W35	<u>W40</u>	<u>CW3</u>	CW4	♂	30
week 42	2	<u>2</u>	11	W15	<u>W40</u>	—	CW4	♂	33
	3	1	<u>2</u>	8	<u>W40</u>	—	NT	♂	28
	4	1	28	W17	W22	—	CW1	♂	—1
	5	9	11	5	W21	—	CW1	♂	—1
	6	<u>2</u>	W31	<u>12</u>	W35	—	CW4	♀	0
	7	1	<u>2</u>	8	W22	—	—	♀	—1
	8	—	11	—	27	—	CW1	♀	—3
	9	11	W26	W16	W35	—	CW4	♀	—2
	10	1	W31	8	<u>W40</u>	—	<u>CW3</u>	♀	—1
Positive control (donor)	<u>2</u>	<u>2</u>	<u>12</u>	<u>W40</u>	—	<u>CW3</u>	♂	♂	60
(Expt 2)									
week 52	11	1	2	8	W40	—	<u>CW3</u>	♂	44
	12	<u>2</u>	3	7	12	—	—	♂	47
	13	3	W30	W35	<u>W40</u>	—	NT	♂	9
	14	<u>2</u>	3	7	<u>W40</u>	—	NT	♀	4
	15	<u>2</u>	3	7	<u>W40</u>	—	NT	♀	—1
	16	<u>2</u>	3	7	<u>W40</u>	—	NT	♀	3
	17	<u>2</u>	9	5	<u>W40</u>	—	<u>CW3</u>	♀	15
	18	1	2	8	<u>W40</u>	—	<u>CW3</u>	♀	6
	19	1	<u>2</u>	5	8	—	—	♀	—1
	20	<u>2</u>	3	7	12	—	—	♀	3
	21	—	9	12	<u>W40</u>	—	<u>CW3</u>	♀	6
	22	11	W31	W15	W17	—	<u>CW3</u>	♀	—1
Positive control (donor)	<u>2</u>	<u>2</u>	<u>12</u>	<u>W40</u>	—	<u>CW3</u>	♂	♂	48
(Expt 3)									
Week 72	23	—	3	18	27	CW2	CW4	♂	—3
	24	1	9	8	27	—	CW1	♂	0
	25	—	3	W17	18	—	—	♂	—3
	26	1	<u>2</u>	5	5	—	CW4	♀	—2
	27	<u>2</u>	W32	8	W15	—	<u>CW3</u>	♀	—3
	28	3	W26	—	W35	—	CW4	♀	1
	29	10	28	W35	HR	—	CW4	♀	0
	30	—	9	7	W15	—	<u>CW3</u>	♀	1
	31	1	3	W25	<u>W40</u>	CW2	CW4	♀	2
	32	1	9	18	W35	—	CW4	♀	1
	33	1	3	—	W35	<u>CW3</u>	CW4	♀	—1
	34	1	28	5	8	—	—	♀	—3
	35	3	W30	7	W17	—	<u>CW3</u>	♀	—3
	36	1	9	8	<u>W40</u>	—	<u>CW3</u>	♀	0
Positive control (donor)	<u>2</u>	<u>2</u>	<u>12</u>	<u>W40</u>	—	<u>CW3</u>	♂	♂	13

NT, not tested.

*The HLA antigens compatible with patient Reef are underlined.

In several cases positive reactions were observed. The level of killing seemed to decline with time, and the gradual waning seemed to be associated with the patient's recovery. Family segregation studies were performed on the relatives of positive panel members. In three such studies the killing pattern failed to segregate with the HLA haplotype, again confirming that killing was directed to a non-HLA target or targets (Table 3).

We have searched for HLA restriction of killing, and a subsequent retrospective analysis revealed a strong association with HLA-A2. All targets that were killed carried the HLA-A2 antigen, although not all HLA-A2 cells were killed. The possibility that a split in HLA-A2 specificities was being recognised was excluded by the family studies (families 2 and 3, Table 3). Here the HLA-A2-bearing haplotype was associated with killing when expressed in one cell, but when expressed on another cell this haplotype was not associated with killing (family 2—c haplotype, family 3—a haplotype). Also, in family 3, no killing was associated with the antigen HLA-A28 which by serology was highly cross-reactive with HLA-A2.

Further analysis revealed that 15 out of 17 targets that were killed were male. Target cells from all fifteen of the HLA-A2 males were killed (Table 4) but cells from males not carrying HLA-A2 were not killed. Cells derived from two females were killed—both females were also HLA-A2 positive, but the level of killing was at a lower level than that observed with other positive reactions in the same test. This suggested that at least two specificities were involved, one associated with the Y chromosome exclusively and the other carried by a proportion of A2 females. One of the females that gave target cells showing positive killing was a sister of the patient (Table 1). The presence of the second system in the males will have been masked by the killing specific for the Y antigen. A clear example of the independent inheritance of an HLA-A2 gene product and the presumed Y product was shown in family 3 (Table 3). Here the HLA-A2 specificity inherited by the male child C1 was from

Table 4 Y chromosome linkage of non-HLA target and restriction of killing to HLA-A2 positive targets

CML	Sex of HLA-A2 targets		
	+	♂ 15 0	♀ 2* 17

*Level of killing was much lower than other positive targets in same experiment.

the mother's a-haplotype. Cells from the two other male children were not killed.

These results demonstrate that the cell-mediated cytotoxic reactions of the patient's lymphocytes are specific for non-HLA antigens but restricted by an HLA product in as much as only targets carrying HLA-A2 are lysed. This is very similar to the restriction seen with murine cytotoxic T cells reacting with non-H-2 antigens¹⁻⁶.

We have not yet unambiguously identified the effector mechanism in this human cell-mediated cytotoxicity system but feel it is not an antibody-dependent system as the cytotoxic reactions occurred in 20% heat-inactivated human AB serum. In these conditions it has been impossible to measure antibody dependent cell-mediated cytotoxicity (L. Doyer, personal communication). This is important as serum from the patient Reef, when tested against the panel members (Table 2) using the two-colour fluorescence technique⁸, gave a positive reaction with some target cells that were positive in the cytotoxicity test.

Our observations of male-associated killing are analogous to those of Gordon *et al.* in the mouse^{5,6}. In their case, cytotoxicity was only observed after *in vivo* sensitisation with a male skin allograft followed by *in vitro* priming. The clinical relevance of the Y-associated killing phenomenon is that it is probably an *in vitro* reflection of the known influence of sex on the incidence of graft against host disease in bone marrow transplantation between HLA identical siblings⁹. In kidney grafting, several workers have observed that female recipients of male grafts survived longer than female recipients of female grafts (ref. 10, and Kissmeyer-Nielsen, Opelz and Terasaki, personal communication). This was interpreted by Oliver to indicate that the Y-linked histocompatibility antigen may actively induce a prolongation of renal allograft survival in man. Although these results contrast with our own, they should be interpreted with caution as HLA-A2 positive combinations were not separately analysed, and some modes of immunisation to the Y-linked histocompatibility antigen may result in protective immunity.

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Table 3 Pattern of killing of a non-HLA target by effector cells (raised between the patient Reef and her donor) tested on 3 families

Family 1 (week 64)	HLA haplotypes	Sex	% Kill
Target			
Mother	ab 1, 8, W6/2, W22, W6	♀	0
Father	cd 1, 8 W6/2, <u>W40</u> , W6	♂	23
C1	ac	♀	0
C2	ad	♂	19
C3	bc	♂	23
Family 2 (week 64)			
Mother	ab 3, 7, W6/1, 8, W6	♀	—1
Father	cd <u>2, 12</u> , W4/2, <u>W40</u> , W6, CW4	♂	42
C1	ac	♂	34
C2	bc	♀	5
C3	ad	♂	41
C4	ac	♀	1
C5	bc	♀	3
Family 3 (week 72)			
Mother	ab <u>2</u> , <u>W40</u> , W6, <u>CW3/28</u> , <u>12</u> , W4	♀	1
Father	cd 1, 8, W6/2, W35, W6, <u>CW4</u>	♂	13
C1	ac	♂	15
C2	bc	♂	—1
C3	bc	♂	—2

In family 2 the female C2 inherited the A2 from the father and was not killed, but her brother who inherited the same A2 was killed. In family 3 the male C1 inherited the A2 from the mother and was killed, but C2 and C3 did not inherit A2 and were not killed. The weeks represent the time after bone marrow grafting that the family studies were performed. Compatibility with patient is underlined.

Selective cellular natural killing against human leukaemic T cells and thymus

NATURALLY-occurring antibodies against thymocytes, certain leukaemias and other tumour cells have been observed in various species including man^{1,2}. In addition, natural cellular immunity—also known as cellular natural killing (CNK)—has been described recently in man³⁻⁵ and in mice⁶⁻⁹. The possible role of CNK in tumour surveillance and the nature of the effector cells are presently subject to extensive research. Relatively little is known, however, about the nature and sensitivity of the target cells in relation to CNK. We report here that human leukaemic T cells and human thymocytes are highly sensitive to CNK by normal human peripheral blood and that B cell lines are relatively insensitive. The response seems to be unrelated to HLA and expressed variably by different individuals.

Tumour cells with T- and B-cell characteristics as described in Table 1 were cultured in minimal essential medium (MEM)

Table 1 Characteristics of tissue cultured human lymphoid cell lines

Cell line	HLA phenotype	Cell type	Cell origin	References
SB	1,2,12,27	B	Leukaemic	10
HSB	1,2,12,27	T	Leukaemic	10
CCRF-CEM	1,w33,6,8	T	Leukaemic	11
JY*	2,7	B	Normal	—
PY*	2,7	B	Normal	—
HL*	2,w35	B	Normal	—
TLCL 23-12-1†	w31,w24,5,w40	B	Normal	—

*Cell lines originally established in culture and kindly provided by Dr D. Mann.

†Cell line originally established in culture and kindly provided by Dr R. Gatti.

supplemented with 10% foetal calf serum (FCS). HSB and SB were obtained from the same individual; HSB is a leukaemic T-cell line, SB is a B cell established from the patient's peripheral blood leukocytes (PBL) in culture. CCRF-CEM is another leukaemic T cell and JY, TLCL, PY and HL are established B-cell lines. Thymocytes were obtained from patients undergoing open-heart surgery. The cell lines and phytohaemagglutinin (PHA) stimulated thymocytes were labelled with ⁵¹Cr and used as target cells in a 4-h modified microcytotoxicity assay⁷. Mononuclear cells from normal individuals were separated from heparinised whole blood on Ficoll-Hypaque as described by Boyum¹² and served as effector cells. To test whether human T- and B-cell lines differ in their susceptibility to lysis, they were labelled with ⁵¹Cr and incubated with normal PBL. Table 2 summarises the results of one such experiment showing that three donors of differing HLA phenotype exhibited high cytotoxic activity against the leukaemic HSB and CCRF-CEM T-cell lines in all ratios tested and had comparatively low activity against SB and JY

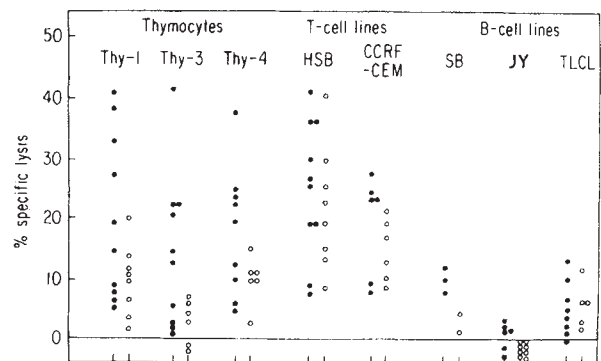


Fig. 1 Distribution of CNK of 18 individuals tested against thymocytes, T- and B-cell lines. A total of 18 samples of human mononuclear cells were tested in several experiments against ⁵¹Cr-labelled PHA-stimulated thymocytes of three individuals (Thy-1, Thy-3 and Thy-4), against two T-cell lines (HSB and CCRF-CEM) and against three B-cell lines (SB, JY and TLCL). The ratio of effector cells to targets was 50:1 in all experiments. Activity is expressed as % specific lysis in a 4-h assay, performed as for Table 2. ●, Male donors; ○, female donors.

B-cell lines. This 'cross-reactivity' is discussed in more detail below. Although HSB and SB were obtained from the same individual and are therefore identical for their HLA and presumably for other cell-surface HLA loci (see Table 1), their reactivities in CNK differ markedly. The other B-cell lines studied—TLCL, PY and HL—behaved similarly to SB and JY (data concerning PY and HL are not presented), while CCRF-CEM, the other leukaemic T-cell line tested, was as sensitive to CNK as HSB. CCRF-CEM and HSB are from different and unrelated subjects. Since leukaemic T cells are thought to be thymus derived it was of interest to investigate the sensitivity of human thymocytes as targets in our cytotoxic assay. Fresh thymocytes used as targets on the same day they were obtained from surgery or cryopreserved thawed thymocytes were unsuitable targets owing to poor ⁵¹Cr incorporation and rapid spontaneous release. Prestimulation with PHA (PHA HA 16/17, Wellcome) at 1 µg ml⁻¹ for 3 d or less resulted, however, in a highly viable population of cells incorporating up to 20-fold more ⁵¹Cr than non-PHA-stimulated cultures on day 0. These cells were then used as targets in the cytotoxic assays. Blood samples from 18 normal individuals were tested against thymocytes obtained from 4 HLA different individuals and prestimulated with PHA for 3 d (Thy-1, Thy-2, Thy-3, Thy-4). All donors tested exhibited significant cytotoxic activity against all thymocyte targets. Table 3 shows the results of four representative donors only. The selectivity of the CNK was demonstrated by the lack of activity against JY, a B cell line. It should be noted that all the B cell lines used in this study served as excellent targets when tested with *in vitro* allogeneically sensitised human PBL (A.O., unpublished data). To our knowledge this is a unique and first example demonstrating CNK against 'normal' cells in general and thymocytes

Table 2 Selective CNK activity against T- and B-cell lines

Effector cells	HLA	% Specific lysis*											
		25 : 1	12 : 1	6 : 1	25 : 1	12 : 1	6 : 1	25 : 1	12 : 1	6 : 1	25 : 1	12 : 1	6 : 1
AO	3,w24,12	29.0	24.3	15.8	21.1	15.5	11.6	7.9	6.7	5.5	6.4	4.9	—1.8
HK	28,w24,14,18	31.7	24.0	17.1	22.6	18.9	11.4	8.8	4.8	7.3	5.3	3.3	1.4
BS	3,7,11,14	22.4	16.0	13.5	18.0	15.6	14.8	6.7	3.9	2.1	—2.9	ND	ND
Spontaneous release			(14.3)			(32.7)			(29.7)			(30.9)	

*Target cells were labelled with 100 µCi of ⁵¹Cr for 2 h at 37 °C, washed three times and resuspended in MEM 10% FCS to 1 × 10⁵ per ml. The cytotoxic assay was performed in Cooke round-bottom microtitre plates by mixing 100 µl of effector cells with 100 µl of target cells and incubated for 4 h at 37 °C in a humidified CO₂ incubator. The reaction was terminated by centrifugation at 250g for 10 min and removing 100 µl of supernates from all wells to be counted in a well-type γ-counter. Specific cytotoxicity was calculated as [(E - SR)/Max] × 100 where E is c.p.m. of experimental wells; SR is spontaneous release from targets incubated in medium alone and Max is maximal release from targets after hypotonic lysis. The standard deviation of triplicate cultures was ±2% of the mean. ND, not determined.

Table 3 Lysis of PHA-stimulated thymocytes by normal PBL

Effector cells	HLA	% Specific lysis							
		1,10w37 Thy-1	2,12,29,w15 Thy-2	2,28,w40,12 Thy-3	10,w30,29,13 Thy-4	1,2,12,27 HSB	2,7 JY	2,12,29,T5 AMT1,	3,w24,12 AO
KT	2,5	32.8	27.0	41.7	19.3	26.0	—2.2	ND	ND
AO	3,w24,12	27.5	18.0	20.2	22.6	29.8	—0.1	0.3	—4.7
DBA	2,5,1,7	40.8	12.2	22.3	ND	36.0	0.9	ND	ND
KY	5,9,w26,w40	38.5	ND	22.5	37.8	41.0	1.0	1.4	—3.0
Spontaneous release		(18.8)	(24.8)	(20.0)	(22.7)	(14.3)	(21.2)	(24.5)	(27.8)

Thymocytes from four individuals and PBL of two donors (A.M. and A.O.) were stimulated in culture with PHA at $1 \mu\text{g ml}^{-1}$ for 3 d, then labelled with ^{51}Cr and used as target cells in the cytotoxic assay. Assay as for Table 2. The ratio of effector to target cell was 50:1 throughout.

in particular. Normal human PBL from two individuals (A.M. and A.O.) stimulated by PHA *in vitro* were not lysed by lymphocytes of normal individuals, confirming an earlier observation³. By screening normal individuals for their activity in CNK it became obvious that different values of specific lysis are obtained with different donors. Figure 1 illustrates this variability when normal donors were tested against three thymocyte populations, two T-cell lines and three B-cell lines. Using thymocytes as targets, cytotoxic activity was 0–42%, compared with 10–40% with the T-cell lines and < 0–15% with the B-cell lines. It seems, therefore, that lymphocytes from different individuals have different killing activities. The range of lytic capacity extends from low to high and does not seem to fall into distinct categories. The difference in the killing activity between males and females seems to be distinct only when thymus target cells are used with males being more reactive. We have noticed that individuals with high reactivity against thymus and T-cell lines occasionally show 'cross-

reactivity' against B-cell lines as shown above (see Table 2). Low killers have a more restricted 'specificity' — killing thymus and T cell lines only.

The nature of the effector cell in CNK is a controversial issue. We have partially characterised them in our system, in limited and preliminary experiments. Different depletion and fractionation procedures resulted in populations of cells with different degrees of CNK activity. Similar profiles were obtained by testing these populations against either thymocytes or HSB target cells. The experiment illustrated in Fig. 2 shows the following. (1) Removal of phagocytic cells by carbonyl iron and magnet enriched the cytotoxic activity against both thymus and HSB targets. (2) Using sheep red blood cells as indicators, the rosetted cells, presumably T cells, were almost inactive. (3) The non-rosetted cells retained their activity. (4) Cells possessing a receptor for the F_c portion of immunoglobulin (F_c+) retained most of the activity against both thymus and HSB cells. And (5), F_c- cells had diminished activity but were by no means depleted of effector function. It therefore seems that the effector cell is not a mature T cell, nor a macrophage but is F_c+ .

We have, therefore, demonstrated cellular immunity of normal individuals against T-cell lines and PHA stimulated thymocytes and a relative lack of activity against B-cell lines even when the T- and B-cell lines came from the same individual. The CNK activity that we observe also does not seem to correlate with HLA. This is in contrast to reports^{14,15} of H-2 specificity in a mouse system. Our results suggesting that males have higher CNK activity than females against thymus cells, are in agreement with the findings of Santoli *et al.*¹⁶ with human (non-lymphoid) cell lines as targets. It is tempting to speculate that our *in vitro* finding of selective CNK against leukaemic T cells but not against B-cell lines may be reflected *in vivo* by the relatively low incidence of acute lymphocytic leukaemias usually associated with cells of the T-axis characteristics¹⁷. Moreover, since T cells are known to be poor antigenic stimulators in MLC¹⁸, it would be logical to propose a different naturally occurring immune mechanism controlling proliferation of immature thymocytes and leukaemic T cells. We suggest that CNK might function in such a surveillance pathway and may also discriminate between mature peripheral T cells on the one hand and immature or leukaemic T cells on the other.

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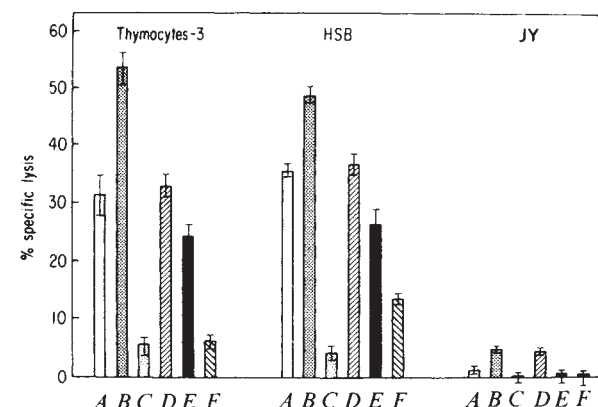


Fig. 2 Partial characterisation of the effector cell in CNK. Normal human mononuclear cells were subjected to various depletion and separation techniques (see below) resulting in populations of cells with different degrees of CNK when tested against ^{51}Cr -labelled PHA-stimulated thymocytes, HSB and JY cell lines. The ratio of effector cells to targets was 25:1. Activity is expressed as % specific lysis in a 4-h assay (as for Table 2). Spontaneous release of the target cells used in this experiment was as follows: Thy-3, 9.3%; HSB, 21.3%; JY 23.8%. A, Normal unfractionated cells; B, PBL depleted of phagocytic cells following ingestion of carbonyl iron ($1 \text{g per } 5 \times 10^6$ cells incubated at 37°C for 20 min); T-cell enriched fraction was obtained by incubating equal volumes of neuraminidase (Behring Diagnostics) treated sheep red cells (SRC) and lymphocytes for 10 min at 37°C followed by a centrifugation at $200g$ for 5 min and incubation in ice for 1 h. The pellet was then gently resuspended in medium and rosetted cells were separated from non-rosetted cells by the use of a Ficoll-Hypaque gradient. C, SRC of the rosetted fraction were lysed with an anti-SRC haemolysin at a 1:10 dilution and fresh undiluted human serum; D, non-rosetted cells with neuraminidase treated SRC after Ficoll-Hypaque gradient separation; E, F_c positive cells were separated from F_c negative cells based on their ability to bind to SRC anti-SRC complexes bound to poly-L-lysine pretreated plastic dishes following the technique of Hsia *et al.*¹³; F, F_c negative cells were obtained by collecting cells not adhering to the antigen-antibody complexes described above.

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Carcinogen-induced mutations at two separate genetic loci are not enhanced by leukaemia virus infection

THE interrelationship between cellular mutagenesis and carcinogenesis has been studied primarily by measuring these two events in separate target systems using chemicals with known biological activity¹. Most carcinogens, in appropriate experimental conditions, induce mutations in prokaryotic² and eukaryotic³ test systems. There are, however, some well known exceptions. Some nucleic acid base analogues and the acridines, which are excellent mutagens, are not known to be carcinogenic *in vivo*. Similarly, asbestoid minerals and some metallic salts which are potent carcinogens do not produce gene mutation. We describe here a study of the relationship between transformation and mutation with different experimental approaches, and show that carcinogen-induced mutations at two separate genetic loci are not enhanced by leukaemia virus infection.

The use of Fischer rat embryo (FRE) cells for *in vitro* assay of chemical carcinogens has been well documented⁴. In appropriate conditions, most of the ultimate carcinogens and procarcinogens transform FRE cells synergistically only after being preinfected with murine leukaemia virus (MuLV)⁷. Since infection by MuLV so profoundly affects the expression of chemically induced transformation in this system, it was of interest to determine the effect of MuLV on the carcinogen-induced cellular mutagenesis in FRE cells.

A clonal subpopulation of the FRE cell line F1706 used in these studies (hereafter designated as M81 cells) has been described previously⁶. Logarithmically-growing M81 cells were infected with 10⁴ XC plaque-forming units of the Rauscher

strain of MuLV (RLV). RLV-infected or uninfected M81 cells were treated with various doses of the potent carcinogen-mutagen, 4-nitroquinoline-1-oxide (NQO), for 1 h, washed and incubated at 37 °C in the growth medium for 72 h (expression time). The post-expression cells from each treatment group (M81+acetone; M81+RLV+acetone; M81+NQO; M81+RLV+NQO) were then used for the determination of DNA polymerase activity⁷, NQO-induced transformation^{8,9}, and mutagenesis at two separate genetic loci.

To determine transformation in M81 cells, a previously described 'horizontal-vertical' assay⁴ which involves both morphologic criterion and the loss of anchorage dependence (soft agar assay) was used. Colonies in semi-solid agar and morphologically transformed foci in liquid medium first appeared in the culture flasks after 5 and 7 subcultures respectively in the dually treated group (RLV and NQO 0.1 µg ml⁻¹). After several more subcultures, the focal characteristic of morphological transformation was lost and the entire culture appeared to be transformed. No morphological transformation or loss of anchorage dependence could be detected in the other treatment groups (M81+acetone; M81+RLV+acetone; M81+NQO 0.1 µg ml⁻¹) before the experiment was terminated at the tenth subculture. The transformation of RLV-infected FRE cells by NQO confirms the results previously reported by other investigators^{10,11}. It should be noted, however, that due to the number of subcultures involved before there was evidence of transformation, enumeration of the foci or the soft agar colony counts should not be used quantitatively.

The number of mutants in M81 cells was determined by plating the post-expression cells at low densities and then, after 72 h, adding appropriate selecting media. For mutation in the recessive locus governing the expression of the enzyme hypoxanthine-guanine phosphoribosyl transferase (HGPRT: EC 2.4.2.8), 8-azaguanine (AZ) was used for selection and all experiments were performed with a single lot of commercial foetal bovine serum. The frequency of the appearance of AZ-resistant colonies per plate followed a typical Poisson distribution ($\lambda = 0.1$) and were verified by χ^2 test ($P > 0.99$). Table 1 shows the number of AZ-resistant phenotypes per 10⁵ survivors in a typical experiment 72 h after chemical treatment (NQO 0.1 µg ml⁻¹, 1 h). A statistically significant increase ($P < 0.01$) over the spontaneous mutation rate was clearly demonstrated with NQO treatment in both RLV-infected and uninfected groups. Using a standard one-tailed test of significance for the observed differences between two Poisson variables¹², however, there was no significant difference ($P < 0.05$) between the number of spontaneous AZ-resistant colonies in the RLV-infected or uninfected M81 cells. Similarly, RLV infection did

Table 1 Transformation and 8-azaguanine resistant phenotypes in RLV⁺ and RLV⁻ post-expression FRE cells

Carcinogen	RLV ⁻		RLV ⁺	
	No addition	0.1 µg NQO	No addition	0.1 µg NQO
DNA polymerase activity	0	0.5	48.6	55.05
Transformation (subculture no.)	— (P ₁₀)	— (P ₁₀)	— (P ₁₀)	+ (P ₅)
No. of replicate plates for AZ-resistance assay	16	20	20	20
% Relative PE (at 72 h)	100	52	188	125
Total no. of AZ-resistant colonies	3	27	8	65
% Plates containing following groups of resistant colonies:				
0	81	40	65	5
1	19	20	30	0
2 or 3	0	30	5	60
4 or more	0	10	0	35
Mean no. of resistant colonies per 10 ⁵ survivors	1.77	19.20	1.39	19

Virion-associated DNA polymerase activity in the cell culture fluids were measured using an established procedure⁷ with synthetic template primer dT₁₂ rA and expressed as pmol of acid-precipitable H³-TMP per h per ml of supernatant collected at 72 h. The input radioactivity used for polymerase assay contained 1930 c.p.m. per picomole of TTP. Transformation assays^{8,9} were performed for 10 subcultures after NQO treatment when the experiment was terminated; results were expressed as either positive or negative at indicated numbers of subculture. For AZ-resistance assay, 1 × 10⁵ M81 cells were plated in 90-mm Falcon Petri dishes 72 h after NQO treatment in EMEM growth medium (Earle's minimal essential medium with 10% newborn calf serum and antibiotics). After 3 d, growth media supplemented with AZ 30 µg ml⁻¹ (selective media) was added. The cultures were subsequently fed every third day with selective media and the dishes were stained with Giemsa on the fifteenth day for enumeration of AZ-resistant colonies. Plating efficiency of each treatment group was determined simultaneously by plating 5 × 10³ post-expression cells in growth media without AZ. These parameters for selection of AZ-resistant cells were optimal for M81 cells in pilot experimentations performed for standardisation of the assay procedure.

Table 2 Reconstruction experiment with HGPRT⁻ subclones

Input cell mixing ratio (AZ-mutants/M81 RLV ⁺)	Carcinogen treatment	% Absolute PE (72 h)	Mean no. of recovered AZ-resistant colonies per 10 ⁵ survivors
0/10 ⁶	None	46.2	1.27
10 AZ-1/10 ⁶	NQO 0.1 µg ml ⁻¹	43.4	18.49
	None	52	25
10 AZ-2/10 ⁶	NQO 0.1 µg ml ⁻¹	49	44.90
	None	56.6	24.91
	NQO 0.1 µg ml ⁻¹	52.8	45.83

5 × 10⁵ AZ-resistant mutants, AZ-1 or AZ-2 were mixed with 5 × 10⁶ RLV infected M81 cells. 2 × 10⁶ mixed cells were plated in T-flasks and incubated at 37 °C. After 6 h, cells were washed and treated in NQO 0.1 µg ml⁻¹ or acetone for 1 h. Each flask was then washed and incubated at 37 °C for 72 h. Selection of the AZ-resistant cells in the mixed population was performed as for Table 1 in order to determine the efficiency of the selection technique.

not significantly alter the outcome of NQO-induced mutation. Thus, although treatment with NQO produced an increase of AZ-resistant cells in both infected or uninfected groups, infection of the target cells with RLV did not alter the spontaneous or induced rate of mutation for the appearance of the drug-resistant phenotype. Several of the AZ-resistant colonies were isolated for further characterisation. Two of these clones, AZ-1 and AZ-2, were propagated for at least 50 generations in nonselective growth medium and still retained their high resistance to AZ (PE 18 and 19.6 respectively and sensitivity (PE < 0.01%) to THMG supplemented growth medium¹⁸. A reconstruction experiment using these two resistant subpopulations was performed to check the validity of the calculations used for the determination of the HGPRT⁻ phenotypes. The results of the reconstruction experiment (Table 2) indicate that the estimates of the proportions of AZ-resistant cells, based on the selection procedure, are within the same order of magnitude of the actual proportions used for mixing resistant cells with the parent M81 population.

Since the phenotypic expression of AZ-resistance can vary

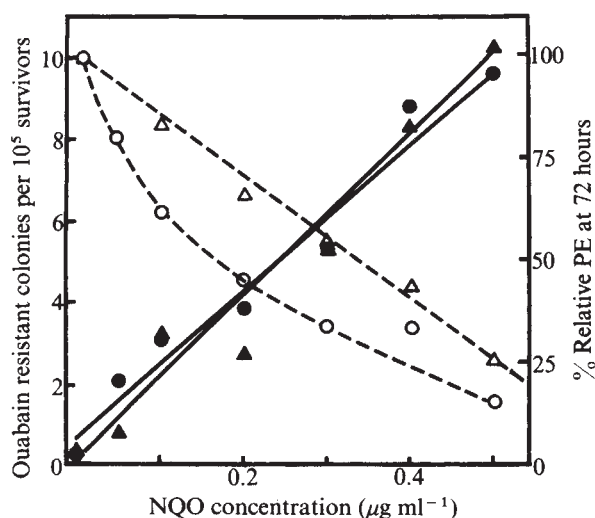


Fig. 1 Percentage survival and ouabain-resistant colonies per survivor in FRE cells as a function of input NQO dose. Δ , M81 relative PE (%); $\circ$, M81+RLV relative PE (%). $\blacktriangle$, Ouabain-resistant colonies in 10⁵ surviving M81 cells; $\bullet$, ouabain-resistant colonies in 10⁵ surviving M81+RLV cells. For ouabain resistance tests, 5 × 10⁵ cells were plated in 90-mm Falcon Petri dishes 72 h after NQO treatment in EMEM growth medium. Ouabain (2.5 mM) was added to the medium after 3 d and dishes were incubated at 37 °C without any subsequent feeding and stained with Giemsa on the fifteenth or eighteenth day for counting the drug-resistant colonies. Each point represents a mean obtained from 10 dishes, except that 20 replicates were used for groups without NQO treatment. It should be noted that normalised relative survival of each test group was based on plating efficiencies obtained at 72 h without NQO treatment (five replicates) and all PEs were performed simultaneously with the ouabain selection procedure. Solid symbols (ouabain resistant colonies) represent means of the experimentally obtained data and solid lines connecting them represent calculated regression lines. See text for regression equations for each line.

several-fold due to minor variations in the experimental protocols, it was considered necessary to determine mutation at another locus. Resistance to the synthetic steroid, ouabain, involves mutation of the gene controlling the expression of cell membrane-bound enzyme Na⁺/K⁺-Mg²⁺-dependent adenosine triphosphatase (ATPase: EC 3.6.1.3). The mutation is co-dominant and a single step selection can be performed over a wide range of input cell concentrations¹⁴. To evaluate the effect of RLV infection on the NQO-induced mutation at the ATPase locus, subconfluent cultures were treated with different doses of NQO and the number of ouabain-resistant phenotypes was determined after 72 h expression period.

Figure 1 shows the dose-response pattern for the appearance of ouabain resistant colonies. It can be seen that over a wide range of NQO input doses, a linear response for the appearance of the ouabain resistant cells is evident in both RLV-infected or uninfected M81 cells. For uninfected cells, the regression line can be best described by the equation $F^- = 19.51 \times \mu\text{g ml}^{-1} \text{ NQO} + 0.27$, while for RLV⁺ cells the linear relationship is $F^+ = 18.2 \times \mu\text{g ml}^{-1} \text{ NQO} + 0.81$, where F^- and F^+ represent mutation frequencies per 10⁵ survivors. However, the slopes of the two regression lines (19.51 and 18.2, respectively) are not significantly different by Student's *t* test ($P \geq 0.05$). Thus, RLV infection again failed to enhance the mutagenic response of M81 cells at any of the NQO doses tested. As before, several ouabain-resistant colonies were isolated, cultured in nonselective medium and these subpopulations were tested in a regular or K⁺-deficient

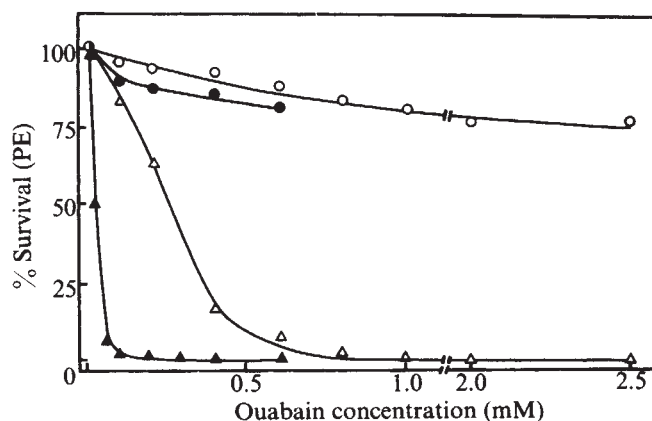


Fig. 2 Relative plating efficiencies of M81+RLV cells and an ouabain-resistant subclone (M418) as a function of ouabain concentration. Δ , M81+RLV cells cultivated in EMEM (55%); $\blacktriangle$, M81+RLV cells in K⁺-deficient EMEM (27%); $\circ$, M418 cells in EMEM (58%); $\bullet$, M418 cells in K⁺-deficient EMEM (49%). The PE of each experimental cell line in ouabain was determined as described in the legend to Fig. 1, except that 10% dialysed calf serum (ds) was used in conjunction with ouabain treatment. Cells were plated at three different concentrations (5 × 10²; 5 × 10³; and 1 × 10⁴ per plate) in triplicate and accordingly zero PE simply reflects that no surviving colonies were observed at the highest plated cell concentration. The average % PE for each of the four experimental groups without ouabain, which has been used as a basis for the normalisation of the data (100%) are given in parentheses.

selective medium for ouabain resistance. The toxicity of ouabain for M81 clones, as manifested by the reduction in the number of surviving colonies, is dependent on K^+ content of the medium. A typical toxicity pattern for one of the resistant subpopulations (M418) is shown in Fig. 2.

Our results indicate that although RLV infection profoundly affects the expression of transformation in FRE cells, mutation rates at two unlinked genetic loci—one co-dominant and the other recessive—are not affected by the virus. Perhaps a specific, privileged role of the ecotropic MuLV on the promotion of cellular transformation at the genetic level can be envisioned which is not applicable to other non-specific and random genetic events like mutation induced by carcinogens. In this context, it is interesting to note that chemical promoters of transformation, such as phorbol esters¹⁵, which presumably act epigenetically, has recently been shown to also enhance the cellular mutagenesis rates¹⁶.

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Antibodies to major histocompatibility antigens produced by hybrid cell lines

FUSION between myeloma cells and spleen cells from immunised donors has been shown to be a successful method of deriving homogeneous anti-SRBC (anti-sheep red blood cell) and anti-TNP antibodies^{1,2}. One of the most powerful features of this approach is that, by cloning, one may easily derive cell lines synthesising monoclonal antibodies despite using non-purified immunogens. The multiple components of a heterogeneous population of hybrid cells are resolved by cloning techniques. This feature makes the system a very powerful tool in the study of complex antigenic structures. The established cell lines offer the further advantage of unlimited permanent supply of material, and the possibility of worldwide standardisation.

We report here the derivation of lines of hybrid cells secreting antibody to rat major histocompatibility antigens. The hybrids were made by fusion of a mouse myeloma cell line¹, P3-X63-Ag8, with spleen cells of AO strain rats immunised against DA strain antigens. In previous experiments we used Sendai virus as a fusing agent. In the experiments described here we used polyethylene glycol (PEG). Fusion of animal cells with PEG is becoming a method of choice. Various protocols have been described in detail, usually for cells growing as monolayers^{3–5}. We adopted some of

these suggestions, which take special care with the rate of addition of reagents, time and temperature of incubations, molecular weight of PEG and so on (see legend to Fig. 1 for details). After the hybridisation the preparation was divided in Linbro plates, each 2-ml well containing about 2×10^6 spleen cells. Vigorous growth was observed in all the wells indicating the presence of multiple hybrid clones in each well. The supernatants of each well were tested for their ability to mediate the complement-dependent lysis of ⁵¹Cr-labelled red cells and lymphocytes from DA strain donors. Of 113 cultures from two independent fusions 107 contained significant lytic activity. The most active cultures were further grown to larger volumes. During growth, some cultures gradually lost activity, whereas others retained or even increased their lytic activity. This presumably reflects the clonal complexity of the cultures—after a certain period, dominant clones (fastest growing) are likely to take over regardless of their antibody activity.

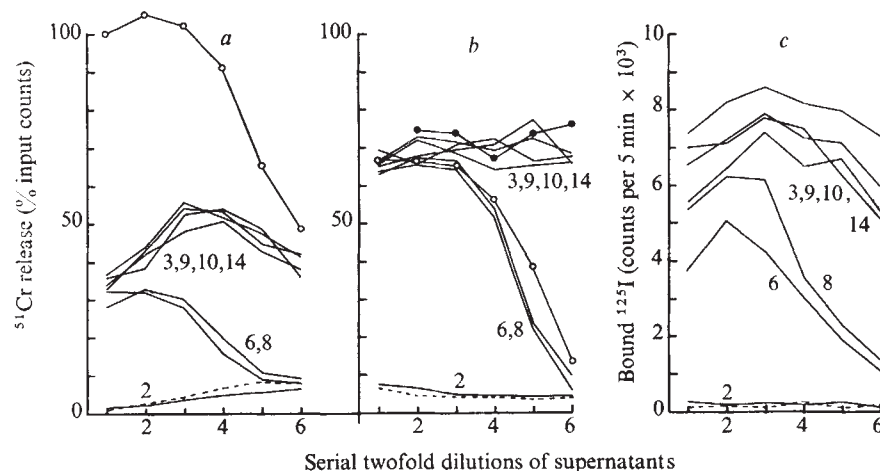
The individual cultures displayed lytic activities which, in some cases, could be shown to be distinctive. For instance, hybrid R2/10 lysed 100% of DA red cells, while R3/13 never lysed more than about 55%, although the titres of the two supernatants were very similar on this target (Fig. 1). Furthermore, the R3/13 supernatants tested for lympholytic activity showed a markedly higher titre than R2/10.

After about 2 months of growth, hybrid R3/12 was cloned in soft agar and 14 individual clones were selected for further studies. Supernatants from six of these were positive in three tests for specific immunological activity, while the eight others were negative in all three tests (Fig. 1). Clones R3/13-6 and R3/13-8 had lower antibody titres than the other positive clones. The Ig produced by the isolated clones was analysed by isoelectric focusing and SDS-polyacrylamide gel electrophoresis of ¹⁴C-lysine-labelled secreted products (Fig. 2). In all cases the characteristic pattern of products of the parental X63 cells due to MOPC 21 IgG1 (κ) was present. All positive clones had very similar patterns of additional bands which clearly established the presence of extra components, presumably responsible for the antibody activity. These extra components were seen in IEF as a few extra bands (Fig. 2) and a background of radioactivity. A similar background of radioactivity has been observed with another plasma cell hybrid (Sp2/HLGK, ref. 2). The presence of the background in this latter case was associated with the presence of a specific IgG2b, an μ globulin which precipitates at low ionic strength (D. Secher, unpublished). Electrophoresis in reducing SDS-polyacrylamide gels shows the presence of an additional light chain in all cases. The putative specific heavy chain co-migrates with the myeloma γ 1 chain but can be separated on much longer runs. A clear indication of clonal heterogeneity was observed in clone R3/13-6 with a weak band in the position of a μ chain and an unusually high molecular weight putative L-chain (Fig. 2). This clonal heterogeneity may explain the low titre of its supernatant (Fig. 1). The reason for the low titre of clone 8 has not been investigated further.

Specifically positive clone supernatants from R3/13 were tested in various ways to demonstrate that the antigenic target for the antibody was associated with the rat major histocompatibility complex, Ag-B. Supernatants were tested against target cells of four inbred rat strains each carrying a different Ag-B haplotype including AO, the strain of the parental spleen cells used for fusion (Table 1). By ⁵¹Cr release from target lymphocytes and by binding to erythrocytes, clone supernatants were reactive only against cells from the original immunising strain, DA. Chromium release data for clone supernatants were compared with two dilutions of a standard AO anti-DA immune serum. This antiserum, which maintains cytotoxic activity at a plateau up to a dilution of about 1/6,000 for DA lymphocytes, cross-reacts detectably on HO targets at about 1/800 and on AGUS targets at about 1/100. Since R3/13 clone product has a plateau cytotoxic titre of 1/384 for DA cells (Fig. 3), detectable cross-reactions would be expected at 1/48 on HO and at 1/6 on AGUS if the specificity of the clone product were similar to that of the AO anti-DA antiserum. The absence of any detectable cross reactions at 1/6 (maximum concentration tested) shows that the R3/13 clone product is much more specific than the immune serum.

Since positive clone supernatants were not reactive against HO

Fig. 1 Immunological activity of spent medium from plasma cell hybrids. Hybrid culture R3/13 was cloned in soft agar and 14 individual clones (R3/13-1 to R3/13-14) removed from the agar and grown separately. Supernatants from numbered R3/13 clones (—) were tested in three assays for immunological activity. Supernatant from the uncloned hybrid R2/10 (○) was included for comparison in two assays. Supernatants from clones R3/13-3, 6, 8, 9, 10, 14 were positive in all three assays, and from clones R3/13-1, 2, 4, 5, 7, 11, 12, 13 were negative in all three assays. Of the negative clones, results are shown only for R3/13-2. DMM served as negative control (---). *a*, Complement-dependent lysis of DA erythrocytes assayed by ^{51}Cr release. 10^8 fresh washed erythrocytes were labelled for 90 min at 37°C with ^{51}Cr -sodium chromate (CJS 4, Radiochemical Centre, Amersham) at approximately 5 mCi ml^{-1} in 0.025 ml . Assays were performed in V-bottomed microtitre trays in a final volume of 0.075 ml consisting of equal volumes of diluted clone



supernatant or medium, washed labelled erythrocytes at a concentration of $5 \times 10^7\text{ ml}^{-1}$ in Dulbecco's phosphate buffered saline (PBS), and guinea pig serum (absorbed with rat erythrocytes) diluted 1:1 in Dulbecco's PBS. Trays were incubated for 60 min at 37°C . After adding 0.1 ml PBS to each well, trays were spun 10 min at $400g$ and 0.1 ml collected from each well for gamma counting. Input counts (32,994 counts per 5 min) were measured from 0.025 ml of labelled erythrocytes at $5 \times 10^6\text{ ml}^{-1}$. Final concentration of supernatant at the first dilution was $1/3$. *b*, Complement-dependent lysis of DA lymphocytes assayed by release of ^{51}Cr . Viable lymph node lymphocytes were separated from erythrocytes and dead cells by centrifugation over Ficoll-Isopaque⁶. Chromium labelling and assay were as described for erythrocytes except that both procedures were performed at room temperature, labelled lymphocytes were diluted to 2.5×10^6 per ml, and the assay was extended to 90 min. Input counts (23,261 counts per 5 min) were measured from 0.025 ml of labelled lymphocytes at 2.5×10^6 per ml. (●), Titration of $(\text{AO} \times \text{HO})\text{F}_1$ anti-DA serum against DA lymphocytes. This serum lyses 100% of DA lymphocytes by Trypan blue exclusion at the dilutions studied in the conditions of this assay. Final concentration of supernatant at the first dilution was $1/3$. *c*, Binding of R3/13 clone products to DA erythrocytes. 1.5×10^6 fresh washed erythrocytes were incubated for 15 min on ice with serial dilutions of supernatants in V-bottomed microtitre trays in a total volume of 0.05 ml . Cells were then washed twice in 0.2 ml PBS and resuspended with 0.01 ml ($180,547$ counts per 5 min) ^{125}I -rabbit F(ab')_2 anti-rat Fab (from Dr A. F. Williams). After a further 15 min on ice, cells were washed five times in 0.2 ml PBS and collected for gamma counting. Final concentration of supernatant at the first dilution was $1/2$. Cell hybrids were prepared as follows: AO strain female rats were hyperimmunised by combined i.p. and subcutaneous immunisations with pooled spleen and lymph node cells from DA strain donors at monthly intervals (approximately $1/3$ donor equivalent per recipient). Two and a half days before collecting spleen cells for hybridisation, hyperimmune AO rats received a final i.v. injection of DA lymphoid cells. Spleen cells for fusion were initially prepared in Dulbecco's PBS containing 2% FCS. The cells were then washed with Dulbecco's modified medium in the absence of serum (medium D), and 10^8 spleen cells mixed with 10^7 P3-X63-Ag8 cells¹ suspended in medium D. The mixture was centrifuged in a 50-ml plastic conical tube at $600g$ for 7 min. The supernatant was removed and the cell pellet disrupted by gently tapping the bottom of the tube. Further operations were performed at about 37°C . A 1-ml pipette containing 0.8 ml of 50% PEG 1500 in medium D (pH 7.6–7.8) was used to suspend the cells gently while the solution was added over a period of 1 min. The suspension was kept at 37°C for 1 min and 1 ml medium D added over a period of another min. A further 20 ml of medium D was added over a period of 5 min. The cells were centrifuged and gently resuspended in Dulbecco's modified medium containing 20% foetal calf serum (DMM). The suspension was distributed in $48 + 2\text{ ml}$ wells in Linbro BCL-5041 trays. In some experiments $3 + 10^3$ untreated spleen cells were also added to each well to serve as a feeder. After 24 h half of the medium was replaced with HAT⁶ medium. This operation was repeated in the two subsequent days and then every 2 d. Vigorous growth in a well after 14 d was taken as a successful hybrid clone(s). These were transferred into HAT medium lacking aminopterin for 4–7 days and thereafter into DMM.

(Ag-B5) cells, it was possible to show that the antigenic target for R3/13 antibody was specified in or near the chromosome region determining the major histocompatibility complex. The tests were performed on progeny from a breeding programme in which the Ag-B4 haplotype of DA is being backcrossed on to the HO

background. Selected Ag-B5/Ag-B5 homozygotes and Ag-B5/Ag-B4 heterozygotes were used as target cell donors to assay the reactivity of positive R3/13 clone supernatants. The results of binding to erythrocytes and complement-dependent lysis of lymphocytes from these genetically typed donors show clearly that

Table 1 Strain distribution of antigenic target for R3/13 clone products

	AGUS	Donor strain		HO	
		AO	DA		
Input ^{51}Cr Cp5min	18,671	19,698	24,122	18,003	
C' control	2.6	2.0	2.5	2.3	Lymphocyte targets*: ^{51}Cr release as % of input counts
AO anti-DA serum at 1/48	51.7	3.9	71.0	60.8	
at 1/3072	1.9	2.1	66.3	2.6	
R3/13 clone 3 at 1/6	2.9	2.6	65.5	2.4	
6 at 1/6	2.6	2.5	60.4	2.1	
8 at 1/6	2.8	2.4	60.0	2.6	Erythrocyte targets†: ^{125}I rabbit F(ab')_2 anti-rat Fab Cp5min bound. Input cp5min = 100712
9 at 1/6	2.9	2.9	62.7	2.7	
R3/13 clone 2 at 1/8	189	167	104	165	
3 at 1/8	181	210	3,613	208	
6 at 1/8	128	149	3,518	176	
8 at 1/8	240	180	4,027	216	
9 at 1/8	158	174	3,882	166	
10 at 1/8	248	198	4,690	188	
14 at 1/8	144	324	3,995	119	

* Complement-dependent lysis of lymph node lymphocytes from AGUS (Ag-B1), AO (Ag-B2) (syngeneic control), DA (Ag-B4) and HO (Ag-B5) donors prepared and labelled with ^{51}Cr - NaCrO_4 as in Fig. 1a, b. Data extracted from full titrations of supernatants and serum performed as in Fig. 1. C' control contained DMM (1/6) instead of spent medium. AO anti-DA serum cross-reacts with AGUS and HO.

† Binding of clone supernatants to erythrocytes from AGUS, AO, DA and HO donors assayed by the second stage binding of ^{125}I -rabbit F(ab')_2 anti-rat Fab as in Fig. 1c.

the antigenic target of the R3/13 clone product is specified by a gene closely linked to Ag-B (Fig. 3).

The antigenic target of clone R3/13 is present on erythrocytes and on all lymphocytes of the DA strain. Furthermore, it has been shown (unpublished results) to be inherited with the main Ag-B4 serological specificities in the only described rat MHC recombinant⁷. These observations and the restricted strain distribution suggest that the R3/13 clone product recognises a K/D-like private specificity of the Ag-B4 haplotype. We presume that analysis of further clone products will enable other specificities of the Ag-B4 haplotype to be identified.

A remarkable feature of the lymphocytotoxic results in the linkage experiment (Fig. 3) was that the R3/13 clone product released only about 45% as much ⁵¹Cr from the Ag-B5/Ag-B4 heterozygous cells and from the (HO × DA)F₁ as from cells of the DA strain, with a marked plateau at this level, suggesting that the target specificity in the Ag-B4 haplotype is weakly expressed, if at all, in about half the lymphocytes from a heterozygote. This phenomenon is not seen with an immune serum (unpublished results). Whether there is total exclusion of one allele for this individual specificity, or whether its expression is being modulated by other elements remains to be seen.

The results described here extend the use of fusion techniques for the derivation of permanent lines producing homogeneous antibodies to the increasingly important area of transplantation antigens. It is hoped that such antibodies will be invaluable in the precise definition of the rat MHC, in study of the expression of its components, and in analysis of functional properties of alloantibodies such as, for example, their role in enhancement. The antigenic target of clone R3/13 is the first uniquely defined antigenic specificity in the MHC. We suggest that this approach should eventually provide the basis for a standard MHC nomenclature. The derivation of a rat-mouse hybrid cell line

Fig. 2 Analysis of the products secreted by isolated plasma cell hybrid clones from R3/13. Cells were incubated with ¹⁴C-lysine and the supernatants analysed as before². *a*, Isoelectric focusing (pH 5, bottom; pH 9, top). *b*, Isoelectric focusing after reduction to separate the chains. *c*, SDS-polyacrylamide gel (10%) electrophoresis after total reduction. X refers to X63 control supernatants. Numbers refer to clones from culture R3/13 (see Fig. 1). O, origin; G and K, γ and κ chains from the Ig secreted by X63 cells; μ, putative μ chain referred to in the text.

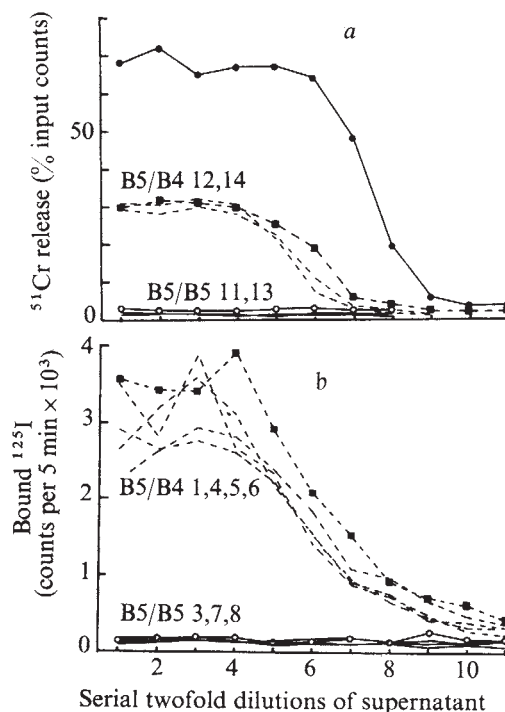
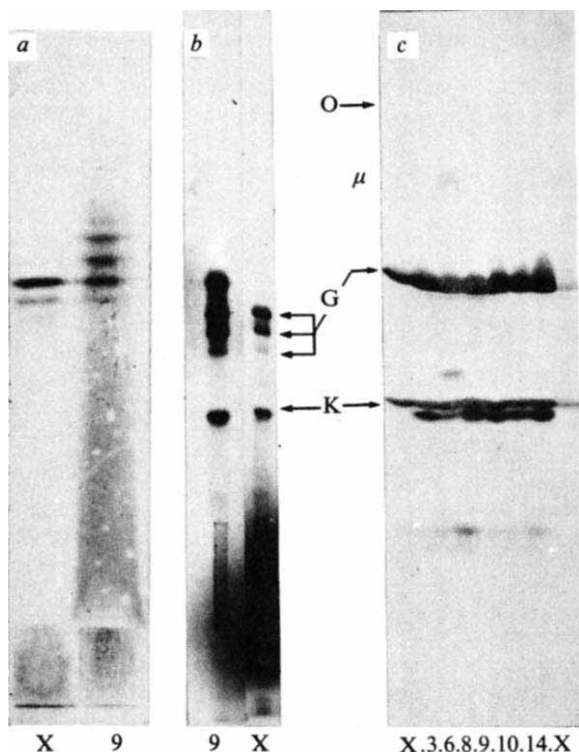


Fig. 3 Linkage of antigenic target of R3/13 clone products to Ag-B. Individuals from progeny of the fifth backcross of the Ag-B4 of DA onto the HO (Ag-B5) background were typed as either Ag-B5/Ag-B4 heterozygotes or Ag-B5/Ag-B5 homozygotes by conventional procedures⁷. Cells from typed individuals were then tested in two assays for the activity of the R3/13 clone product. *a*, Complement-dependent lysis of lymph node lymphocytes by R3/13 supernatant, assayed by release of ⁵¹Cr. Lymphocytes were prepared, labelled and assayed as for Fig. 1b. Cells from DA (●), (HO × DA)F₁ (■) and the Ag-B5/Ag-B4 heterozygotes 12 and 14 (---) were lysed, while cells from HO (○) and the Ag-B5/Ag-B5 homozygotes 11 and 13 (—) were unaffected. Input ⁵¹Cr counts for the seven populations tested ranged from 23,066 to 30,521 counts per 5 min. Final concentration of supernatant at the first dilution was 1/12. *b*, Binding of R3/13-9 clone product to erythrocytes. The binding of clone product was assayed indirectly as for Fig. 1c, by the subsequent binding of ¹²⁵I-rabbit F(ab')₂ anti-rat Fab. Supernatant was bound to erythrocytes from the (HO × DA)F₁ (■) and the Ag-B5/Ag-B4 heterozygotes 1, 4, 5 and 6 (---) but not to erythrocytes from the HO (○) and the Ag-B5/Ag-B5 homozygotes 3, 7 and 8 (—). Input ¹²⁵I counts were 113,077 counts per 5 min. Final concentration of supernatant at the first dilution was 1/4.

producing antibody against a rat transplantation antigen brings the goal of producing standard permanent supplies of monoclonal antibodies for human tissue typing and other clinical uses one step nearer.

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Neurite induction in mature cortical neurones in feline G_{M1} -ganglioside storage disease

PYRAMIDAL neurones of the human cerebral cortex develop spine-bearing structures (meganeurites) between the cell body and the initial portion of the axon in lysosomal storage diseases characterised by intraneuronal accumulation of gangliosides¹. Meganeurites attain enormous proportions and acquire bizarre shapes, suggesting that their development involves restricted reactivation of embryonic growth mechanisms of the neurone. Because outgrowth of neurites is a sign of differentiation in embryonic neurones we have sought evidence for neurite outgrowth from meganeurites in feline G_{M1} -ganglioside storage disease²⁻⁴. We report here that in this system meganeurites of mature cortical neurones regularly exhibit extensive secondary outgrowth of neurites.

Brain tissue was obtained from an 11-month-old mutant cat with confirmed β -galactosidase deficiency and abnormally high levels of the monosialoganglioside G_{M1} -ganglioside in the brain². The cat was killed with pentobarbital sodium near the terminal stage of the disease. This enabled us to obtain well preserved neurones and their processes for Golgi studies¹. The brain was then perfused with a mixture of glutaraldehyde and paraformaldehyde for morphological studies.

Aberrant growth processes were observed in several varieties of cortical neurone. Most small and medium-sized pyramidal neurones of the cerebral neocortex had large pleomorphic meganeurites between the base of the perikaryon and the initial axonal region, as in human G_{M2} -gangliosidosis¹. But the meganeurites in the feline G_{M1} -gangliosidosis had morphological features rarely observed in the human condition, in particular many fine neurites arising from them. These outgrowths had morphological features related to the cell type and characteristics of the relevant meganeurite (Fig. 1).

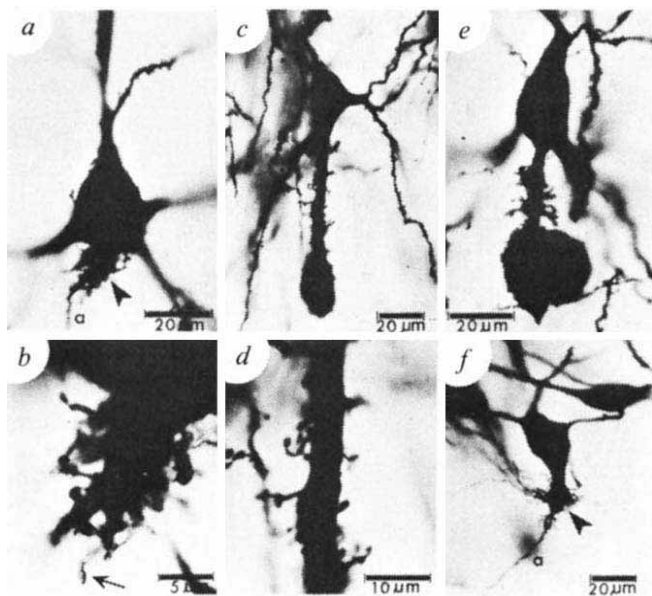


Fig. 1 Rapid Golgi preparations of cortical neurones in feline G_{M1} -gangliosidosis. *a*, Medium-sized pyramidal cell of the entorhinal cortex, meganeurite identified, at arrowhead. *b*, Enlargements of meganeurite in (*a*) to show tangle of neurites one of which has an expanded terminal head (at arrow). *c*, A neurone with a long meganeurite that gives rise to multiple neuritic growth processes, seen at higher magnification in (*d*). *e*, Another small pyramidal neurone of the cerebral cortex has a meganeurite that ends in a huge globular process. Outgrowth of neurites is prominent in the connecting stalk of the meganeurite. *f*, Granule cell of the fascia dentata of the hippocampus. Meganeurite, at arrowhead, exhibits extensive neuritic outgrowth (a, axon).

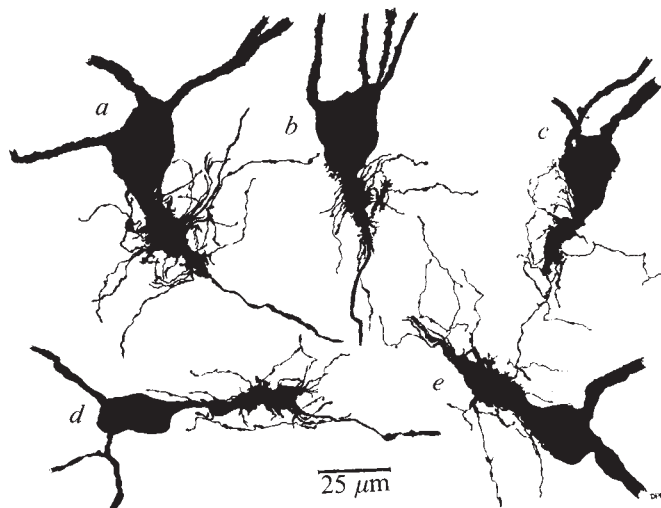


Fig. 2 Camera lucida drawings of granule cells of the fascia dentata. Only the proximal dendrites of these elements are illustrated. Cell bodies show variable shape distortion. Meganeurites arise from the base of the cell body and displace the axon distally. Extensive neurite proliferation and other growth processes are evident. Axons are identifiable in cells (*a*), (*b*) and (*d*). Fine processes also occur on portions of the cell bodies of neurones (*b*), (*c*) and (*e*).

A pyramidal neurone of the entorhinal cortex is shown in Fig. 1*a* with a meganeurite emerging from the base of the cell body. Many neuritic processes are shown as tangled outgrowths from the meganeurite, often with expanded terminal heads (Fig. 1*b*). A wide variety of neurites and other growth processes (spinules, spicules, filopodia-like elements) occur on larger meganeurites of small pyramidal neurones (Fig. 1*c*, *d* and *e*).

Extensive development of neurites on meganeurites was particularly clear in granule cells of the fascia dentata. In this relatively homogeneous population of small neurones, meganeurites formed large fusiform extensions from the base of the cell body, which is normally devoid of processes other than an axon. Long thin neurites appeared as tangled outgrowths from the meganeurite (Fig. 1*f*). The 'medusa-like' characteristics of these outgrowths is illustrated in camera lucida drawings of fascia dentata granule neurones (Fig. 2). In each of these elements the meganeurite is the site of extensive secondary neurite production. Neurites exhibit beading and tufted expansions, which are common features of neuronal growth processes in Golgi preparations⁵.

G_{M1} -gangliosidosis is associated with the development of meganeurites on many types of cortical neurones in the cat, as is G_{M2} -gangliosidosis in man. Although secondary neurite formation has rarely been observed on meganeurites in classical Tay-Sachs disease¹ (infantile G_{M2} -gangliosidosis, B variant) accumulation of G_{M1} -ganglioside in feline cortical neurones seems to be a powerful stimulus for the outgrowth of secondary neurites from meganeurites in the feline mutant. Golgi studies of neurones in human G_{M1} -gangliosidosis will be necessary to determine whether the neurite proliferation is uniquely related to G_{M1} -ganglioside accumulation or the specific response of feline neurones to the ganglioside.

The demonstration that meganeurites in feline G_{M1} -gangliosidosis have growth characteristics typical of differentiating embryonic neurones raises the question of the role of gangliosides in neuronal development and synaptogenesis. Their distribution and content in the brain change during development and they are found in high concentrations in synaptosomes⁶. Although butyrate-induced process formation in differentiating HeLa cells is associated with an increase in G_{M3} -ganglioside⁶, previous studies have not suggested specific involvement of gangliosides in neurite induction in normal embryonic neurones. Our results link G_{M1} -ganglioside accumulation to neurite

formation in well-differentiated cortical neurones in an inherited metabolic disorder. It will be interesting to determine whether gangliosides induce or facilitate process formation in normal immature mammalian cortical neurones *in vitro*.

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The neural representation of visual space

THE approximate form of the projection of visual space on the striate cortex in man has long been established from neurological evidence^{1–3} and estimates of cortical magnification M (the extent of striate cortex in millimetres corresponding to a degree of arc in visual space) have been derived from studies on cortical phosphenes and visual acuity⁴, and migraine scotoma dimensions⁵. The possibility that M could be estimated from the density of retinal ganglion cells which provide the output from the eye to the brain has received support from studies on monkeys^{6–8}. It has been shown that M is proportional to $\sqrt{D_c}$ (where D_c is the projected ganglion cell density in cells per solid degree of visual space) for peripheral angles (θ) greater than 10° . More centrally, where D_c is maximal, this relationship breaks down because the cells are displaced from their receptive fields by an amount which is difficult to determine⁸. If data on ganglion cell receptive field density, D_r (in receptive fields per solid degree) were available, they might be expected to relate to M at every point in the visual field. I report here that I have obtained such estimates of D_r and examined their usefulness as predictors of M . The results are summarised in three basic equations.

In previous studies on human retinal ganglion cell density it was assumed that solid angles were directly related to retinal areas^{9–11}. Subsequently, it was reported that even at retinal level the projection of the visual field is progressively compressed towards the periphery¹². Errors in estimates of D_c , due to the outward projection of an area of retinal section, and its location in the field could easily approach 100%. To avoid these, the original data from three studies on twelve human eyes (refs 9–11) were corrected by use of a specially designed schematic eye¹² which also diminished the errors arising from tissue shrinkage and sampling variations in ocular dimensions. The reprocessed values of cell density expressed in the form $1/\sqrt{D_c}$, plotted against θ , are shown in Fig. 1. This mode of display, originally popularised by Weymouth¹³ is crucial to the argument by which the values of $1/\sqrt{D_c}$ are transformed to values of $1/\sqrt{D_r}$. As M is believed to be proportional to D_r , the receptive field curve affords the possibility of estimating M at any peripheral angle θ . This relationship can, however,

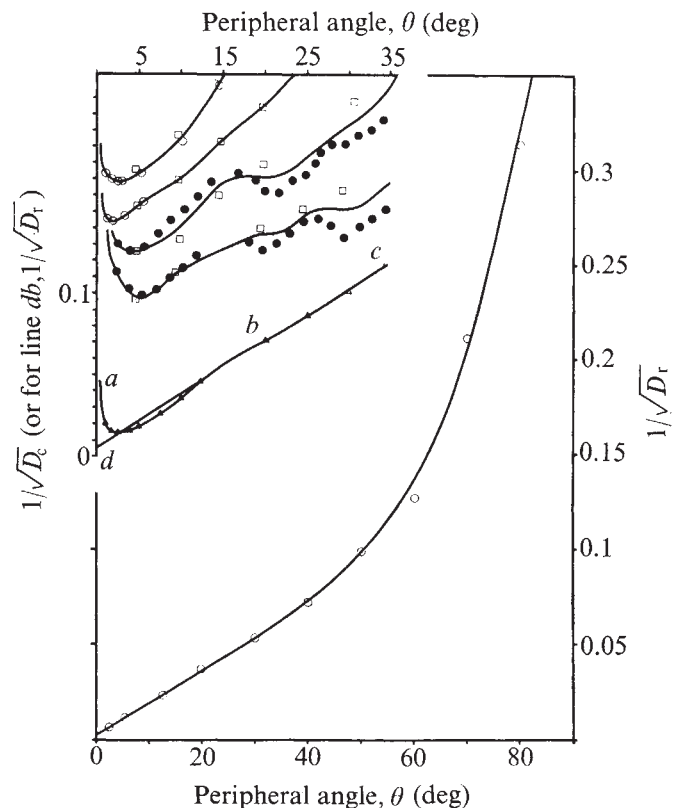


Fig. 1 The curve *abc* relates the value $1/\sqrt{D_c}$ (where D_c is the number of ganglion cells per solid degree) for the peripheral angle θ . It is the mean of the curves for the temporal nasal inferior and superior meridians of the visual field, sections of which are shown displaced upwards to avoid superimposition. The curves are based on the modified data (see text) of Vilter⁹ $\circ$, Van Buren¹⁰ $\square$, and Opel¹¹ $\bullet$. The curve *dbc* is similar but applies to receptive fields instead of ganglion cells. D_r is the receptive field density (per solid degree) and the curve relates $1/\sqrt{D_r}$ to θ . It was obtained by interpolating from *b*, beyond the area affected by excavation to *d*, at 0.0055 following Polyak's observations on the separation of the central-most foveal cones which he considered were equal in number to their ganglion cells¹². It was realised that the two curves should determine equal populations of cells and numerical integration of D_c and D_r with solid angle showed only 6% difference in cell counts. The populations could be equated by slightly reducing the intercept value but conversely it would need to be slightly increased to take account of the findings of Missotten of the ratio of 0.9:1 for ganglion cells to cones in the rod free area¹⁵. The initial value was, therefore, retained as the best compromise for the purpose of the regression curve. The lower right coordinates refer to the plotted points of the final $1/\sqrt{D_r}$ values. The fitted curve is the curve of equation (1) when $k = 0.0055$. Integration of D_r with solid angle yielded an estimated total count of 1.5×10^6 receptive fields per eye.

be further evaluated by correlating the $1/\sqrt{D_r}$ values with $1/M$ derived from studies of cortical phosphenes, visual acuity and migraine scotomata.

The minimum angles of resolution obtained from the mean values of Wertheim's data on visual acuity¹⁶, for horizontal and vertical meridians at eight selected eccentricities were compared with the receptive field data at the same points (Fig. 2). The points were chosen to give an appropriate weighting for the numbers of receptive fields within 40° peripheral angle which included more than 85% of the total count. The correlation coefficient with $1/\sqrt{D_r}$ values was very high ($r = 0.9934$) confirming some earlier suggestions^{13,17}. The same approach was used on the data of Frisén and Glansholm for the horizontal meridian, which were obtained by a method which reduces optical degradation¹⁸. Surprisingly, the correlation was not quite so high, a fact which might be attributed to their use of a constant size of task field (4°) as Wertheim had enlarged the task details and field size equally.

The migraine scotoma has a characteristic angular velocity¹⁹

and segment size^{5,20} which varies with peripheral angle and is related to cortical magnification. Data were extracted from a graphical display⁵ of scotoma dimensions and the least squares regression line predicting scotoma bar length from peripheral angle was used to derive best estimates for comparison with the $1/\sqrt{D_r}$ values. The correlation coefficient was again very high ($r = 0.9995$).

The most direct evidence of human cortical magnification was derived from the graphically displayed findings of Brindley and Lewin on visual field phosphenes resulting from cortical stimulation²¹. The angular separation of phosphenes was divided by the linear separation of electrodes to yield a value inversely proportional to cortical magnification. The method of obtaining these values, though completed independently, resembled that of Cowey and Rolls⁴. A regression line again provided the best estimate of the reciprocal of cortical magnification for selected values of θ which were correlated ($r = 0.9992$) with the $1/\sqrt{D_r}$ values (see Fig. 2).

These comparisons clearly show that $1/M$ is almost exactly proportional to $1/\sqrt{D_r}$. Evidently therefore M^2 , the cortical projection of a solid degree is proportional to D_r , the number of receptive fields per solid degree, and the constant which relates them must be the total striate area (for which 6,000 mm² would be an acceptable estimate⁴), divided by 1.5×10^6 , the total ganglion cells of one eye (Fig. 1 legend). For the data of this study therefore, $M^2 = 0.004 D_r$ and since the central foveal value of $1/\sqrt{D_r}$ was found to be 0.0055, D_r must be 33,058 cells per solid degree so that $M^2 = 132$ mm² per solid degree, and $M = 11.5$ mm per degree. This maximal value of M is close to that suggested by Richards⁵, but differs substantially from the 15.6 mm per degree found by Cowey and Rolls⁴. It has been found, however, that the discrepancy diminishes peripherally and the results are not statistically incompatible. Cowey and Rolls used the M values of Brindley and Lewin, and related these to the acuity data of Wertheim to obtain their result. This depended in the similarity of the visual charac-

teristics of two individual subjects. The finding of the present report is clearly based on extensive new data, and probably therefore more reliable.

Due to the near-perfect correlations between the $1/\sqrt{D_r}$ values and other criteria, the following equation not only describes the variation of $1/\sqrt{D_r}$ with θ , but has some remarkably general properties in relation to the structure and functions of the visual system

$$V = k[1 + S\theta(1 + 3\theta^2 \times 10^{-8} + 8(S\theta)^{5.5} \times 10^{-10})] \quad (1)$$

V provides an estimate of $1/\sqrt{D_r}$ or any of its correlates, such as $1/M$ or the threshold of resolution, at a peripheral angle θ if in each case the foveal value is substituted for k . S determines the initial slope of the regression curve but the peripheral slope and the limit of the visual field are controlled by the last two terms. If data averaged for all meridians are considered, $S = 0.59$ and the linear relationship, $V = k(1 + 0.59\theta)$ gives a useful approximation when θ is less than 30°.

The number of receptive fields per solid degree at a peripheral angle θ is easily estimated from equation (1). If $k = 0.0055$ the $1/\sqrt{D_r}$ value at the fovea, V gives $1/\sqrt{D_r}$ at θ from which D_r is deduced. Similarly, if $k = 1/M$ for the fovea, or $1/11.5$ then $V = 1/M$ at θ , and M , and thus M^2 are easily obtained. (In the case of the monocular temporal crescent, however, the estimates of M and M^2 should be divided by $\sqrt{2}$ and 2, respectively.) Again, if k is the foveal threshold of resolution in minutes of arc, V provides the value at θ and thus its reciprocal, the Snellen decimal acuity. The maximum spatial information density in bits per solid degree is then $3600/V^2$, following the logic of simple information models of vision²². Such estimates are, of course, primarily related to the threshold of practiced observers.

The use of equation (1) is extended specifically to the temporal nasal, superior and inferior portions of the principal meridians of the visual field, if $S = (0.46 - 0.00043\theta)$, $(0.50 + 0.0019\theta)$, $(0.62 + 0.0033\theta)$ or $(0.66 - 0.0006\theta)$. The corresponding curves, as with the general form of equation (1), have a short portion which is adequately simulated by a straight line. In this case $S = 0.46, 0.50, 0.62$ or 0.66 and $V = k(1 + S\theta)$. This simple relationship is applicable when $\theta < 35^\circ, 20^\circ, 10^\circ$, and 20° , taking the meridians in the above stated order.

The linear function, $V = k(1 + S\theta)$, for small angles permits the possibility of the following analytical solution to the problem of cumulative estimates:

$$C = \int_0^\theta \frac{2\pi\theta d\theta}{[k(1 + S\theta)]^2} = \frac{2\pi}{(kS)^2} \left[\log_e(1 + S\theta) + \frac{1}{(1 + S\theta)} - 1 \right] \quad (2)$$

This gives a cumulative estimate which is within 1% of the value indicated by this study if $\theta < 10^\circ$, thereafter it becomes less accurate and overestimates by 5% at 45° . The symbols are as in equation (1), thus if K is 0.0055 equation (2) gives an estimate of the total number of receptive fields in a concentric solid angle of visual space, where θ is the angular semi-diameter. Similarly, if $k = 1/M$ for the fovea, that is $1/11.5$, equation (2) gives the area of cortex (mm²) again corresponding to the same concentric solid angle. Due to the nature of the cortical projection, however, areas of less than 100 mm² are best described as a percentage of cortical input.

When the receptive field counts assessed by numerical integration of D_r and solid angle were examined it was noted that the cumulative count can be described by the simplest and perhaps the most useful equation

$$Q = 100[1 - \exp(-0.0574\theta)] \quad (3)$$

where Q gives a close approximation to the percentage of total receptive fields, striate area or information channel

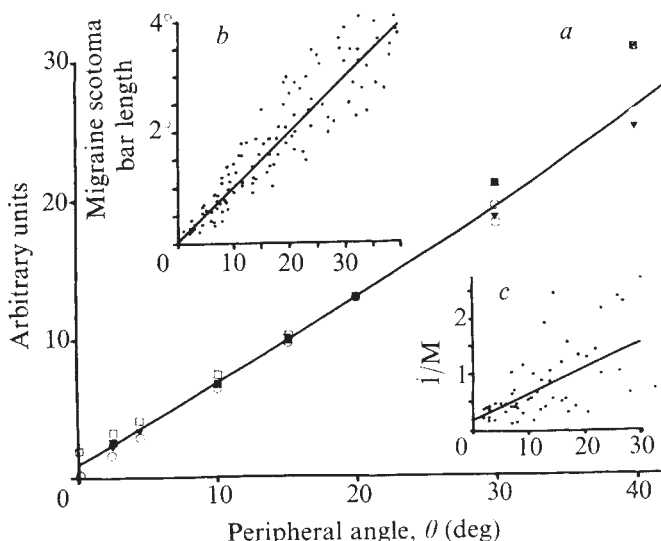


Fig. 2 *a*, Receptive field data ($\blacktriangledown$) and curve plotted for comparison with minimum angle of resolution data ($\blacksquare$), best estimates of migraine scotoma size ($\circ$) and cortical magnification ($\square$). All data are scaled to coincide at 20° where the ganglion cells are unaffected by excavation and where the best estimates of migraine scotoma lengths and cortical phosphene M values are most reliable. Visual resolution at this point is also considered to be relatively unaffected by optical degradation. The origin of the best estimates is shown in (b) and (c) which are based on the data of Richards⁵ and Brindley and Lewin²¹. The main ordinate is scaled in arbitrary units based on the intercept of the receptive field curve which therefore satisfies equation (1) when $k = 1$. The displacement of the receptive field data points relative to the curve as compared with Fig. 1 is due to the rescaling at the 20° point. The graph shows the proportionality between the different criteria of cortical representation which are reflected in the high correlation coefficients quoted in the text.

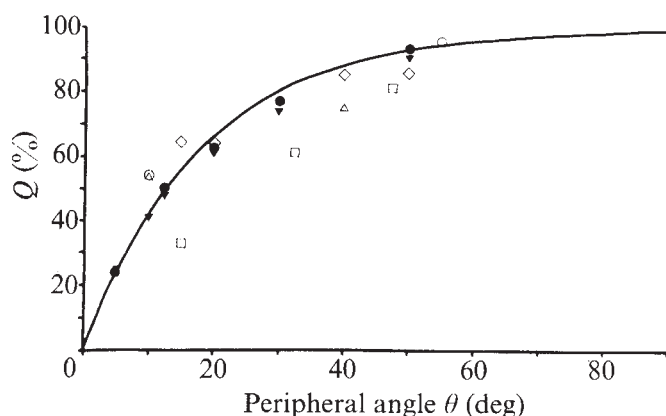


Fig. 3 Cumulative counts of ganglion cell receptive fields provided a method of estimating the percentage of the surface area of the striate cortex corresponding to a concentric region of visual space. Q is cumulative % of receptive fields, cortical area or channel capacity; ●, % receptive fields found in this study; ▼, % using equation (1) instead of actual data, curve is that of equation (3). Additional points derived from the estimates of areas from neurological maps of Holmes¹ □, Spalding² △, Teuber *et al.*³ ◇, and Duke Elder²³ ○ are shown for comparison. Although such charts are designed to convey a quantitative impression, ambiguity is inevitable with the two-dimensional projection of the brain surface. Approximate values were obtained by studying the charts in conjunction with anatomical material and models, and assessing the area of redrawn sections by use of rectangular grids.

capacity for a concentric solid angle of visual space with angular semi-diameter of 6° . The cumulative count and the curve of equation (3) are shown in Fig. 3 and these may be compared with the result of an attempt to relate the present findings to accepted neurological schemes.

Contemporary theories present a complex picture of the neural connections of the visual system, but this does not diminish the usefulness of these equations which merely reflect the quantitative relationships between assembled data and do not depend on a hypothesis involving sensory units.

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Enkephalin may mediate euphoria and drive-reduction reward

THE identification in brain of opiate receptors¹ and morphine-like pentapeptides (methionine-enkephalin and leucine-enkephalin)² has led to suggestions that enkephalin receptors may be sites at which opiate drugs induce analgesia and euphoria, and that the enkephalins themselves may be transmitters or modulators in brain systems for the regulation of pain and pleasure^{3–6}. Observations in animals reveal that appropriate central administration of enkephalin produces morphine-like analgesia^{7–9} and physical dependence¹⁰. We report here that enkephalin also may share the euphorogenic or rewarding properties of morphine in that rats will work for enkephalin injections delivered directly into the ventricles of their own brains¹¹. Of particular interest, in the light of the generally superior potency of the methionine peptide^{2,8,9}, is the observation that leucine-enkephalin is self-administered with greater avidity than methionine-enkephalin.

Guide cannulae, of 19-gauge stainless steel tubing, were implanted above the lateral ventricle in 300–400-g male Charles River rats under pentobarbital anaesthesia. Following a recovery period of at least 1 week, rats were tested for intraventricular self-administration behaviour in a Skinner Box¹². A 25-gauge stainless steel injection needle was inserted in the lateral ventricle via the guide cannula. The needle was connected by polyethylene tubing through a feedthrough swivel device to an infusion pump. The tubing contained one of the substances (dissolved in Ringer's solution and adjusted to pH 5.8) listed in Table 1. Each lever-press response delivered 1 μ l of fluid to the brain in 0.9 s; however, responses made during the 0.9-s infusion intervals had no consequences and went unrecorded. Each rat was tested once and had access to only one solution. No lever-press training was given; rats were merely placed in the Skinner box, with food and water available, for a 66-h session.

The peptides were synthesised at Wyeth Laboratories or purchased from Bachem Inc. After purification by gel filtration (Sephadex G10), homogeneity of the peptides was verified by amino acid analysis and thin-layer chromatography. The use of highly purified material was essential; central administration of unfiltered leucine-enkephalin often caused lethal convulsions. After storage in polyethylene tubing for 66 h, solutions of methionine-enkephalin exhibited a negligible loss of activity in a standard opiate receptor binding assay⁹.

Rates of self-administration were significantly higher for the enkephalins and morphine (but not the structurally related tetrapeptide, Tyr-Gly-Gly-Phe) than for Ringer's solution (Table 1). The order of preference for the peptides when tested at either 1 or 10 μ g per injection was leucine-enkephalin > methionine-enkephalin > Tyr-Gly-Gly-Phe (Fig. 1). Both concentrations of leucine-enkephalin were self-injected at a higher rate than the single dose level of morphine (0.5 μ g per injection), although in neither case was the difference statistically significant. The 1- μ g doses of both pentapeptides caused more rapid learning of self-administration behaviour than the 10- μ g doses, but the 10- μ g doses generated a more sustained performance level (Table 1). Massive dosages of leucine-enkephalin (up to 10 mg in 66 h) were taken by the best responders, despite the possibility of tissue damage or other adverse effects that might have been produced by the passage of large volumes of fluid through the brain ventricles. Indeed, injections of the Ringer's vehicle itself suppressed lever-pressing behaviour. The mean response rate in the Ringer's group fell significantly below that of a non-injected group tested with empty syringes ($P < 0.005$).

To test that nonspecific behavioural stimulation was not

Table 1 Summary data of self-administration experiments

Drug	No. of rats	Dose ($\mu\text{g } \mu\text{l}^{-1}$)	First 24 h	Mean self-administration responses $\pm$ s.e.m.		Highest 6-h rate
				Final 24 h	66-h Total	
Ringer's	25		64.0 $\pm$ 10.7	54.7 $\pm$ 11.3	167.9 $\pm$ 24.3	77.6 $\pm$ 6.2
Morphine sulphate	9	0.5	103.4 $\pm$ 35.2	89.1 $\pm$ 14.4	283.0 $\pm$ 67.1*	54.9 $\pm$ 13.6
Tyr-Gly-Gly-Phe	5	1	71.8 $\pm$ 29.5	96.8 $\pm$ 47.7	237.6 $\pm$ 103.0	51.0 $\pm$ 18.3
Tyr-Gly-Gly-Phe-Met	14	10	74.0 $\pm$ 17.4	85.0 $\pm$ 26.8	221.8 $\pm$ 54.2	56.6 $\pm$ 11.8
	15	1	103.9 $\pm$ 16.5†	120.7 $\pm$ 41.9*	287.5 $\pm$ 70.1*	68.7 $\pm$ 13.4
	12	3.3	93.3 $\pm$ 14.8	86.2 $\pm$ 15.2	252.1 $\pm$ 35.6*	56.8 $\pm$ 9.6
	19	10	79.2 $\pm$ 14.9	105.5 $\pm$ 21.5†	266.6 $\pm$ 50.7*	56.8 $\pm$ 8.1
Tyr-Gly-Gly-Phe-Leu	6	1	141.7 $\pm$ 29.8‡	99.7 $\pm$ 36.8	333.2 $\pm$ 60.9‡	83.3 $\pm$ 12.0‡
	18	10	116.6 $\pm$ 43.2	186.9 $\pm$ 56.4†	432.4 $\pm$ 131.3†	110.3 $\pm$ 28.2‡

*Significantly different from Ringer's solution, $P < 0.05$.

† $P < 0.025$.

‡ $P < 0.005$.

Rats implanted with permanently-indwelling intraventricular cannulae had continuous access to one of the indicated drug solutions in a single 66-h test. Each lever-press response delivered 1 μl of solution into the lateral ventricle in 0.9 s.

responsible for the high response rates in the enkephalin groups, rats previously trained to lever press for food or brain-stimulation rewards were treated with leucine- or methionine-enkephalin (50–200 μg , intraventricularly) just before the behavioural test. Lever-press rates were suppressed rather than facilitated by both peptides (Table 2), and observations of the animals revealed that their activity level had been depressed rather than increased. The behaviour more plausibly may reflect a morphine-like rewarding or addicting action of the peptides. The observed preference for leucine-enkephalin over methionine-enkephalin also may be consistent with a reward interpretation. Opiate receptor binding by the leucine peptide is reduced more by sodium and enhanced more by manganese than is the case for the methionine peptide^{9,13}. Since a similar sensitivity to sodium and manganese differentiates the highly addictive opiate agonists from other classes of opiate drugs¹³ these observations provide independent evidence that leucine-enkephalin may be a 'purer' opiate agonist with greater addictive or euphorogenic potential than methionine-enkephalin.

Why would a natural substance such as leucine-enkephalin possess addictive properties? One interesting, if highly speculative, possibility is that a peptide similar or identical to leucine-enkephalin may serve as a natural euphorigen⁶ or reward transmitter. If this conjecture were true, reward or addiction should also be produced by release

of endogenous peptide following electrical activation of enkephalin-containing neurones in the brain. Behavioural and immunohistochemical observations may be consistent with this prediction. Sites that yield high rates of self-stimulation¹⁴ and those that contain dense networks of enkephalin-like immunoreactivity¹⁵ often overlap in precisely the same brain regions (for example, pontine central grey, zona compacta of the substantia nigra, bed nucleus of the stria terminalis and nucleus accumbens). According to our hypothesis, self-stimulation of these regions would depend at least in part on the electrically-induced release of enkephalin and the consequent activation of opiate 'reward' receptors. If so, the behaviour should be suppressed or extinguished following administration of an opiate receptor antagonist such as naloxone.

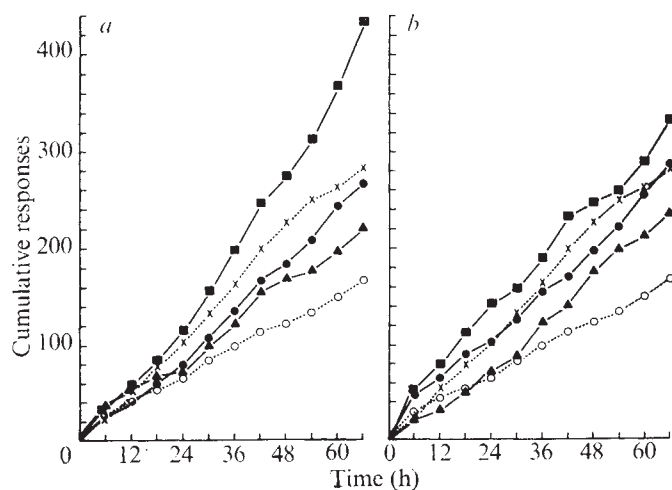
Table 2 Suppressant action of enkephalin on operant behaviour maintained by food or brain-stimulation reinforcement

Drug	Dose (μg)	Lever-press rate (% of no drug)			
		Food	N	Brain stimulation	N
Ringer's		125.5 $\pm$ 23.4	4	101.8 $\pm$ 12.4	9
Methionine-enkephalin	50	57.6 $\pm$ 25.6	4	not tested	
	100	46.1 $\pm$ 35.9	4	84.3 $\pm$ 5.2	3
	200	not tested		38.8 $\pm$ 28.6	2
Leucine-enkephalin	100	57.8 $\pm$ 16.1	8*	61.1 $\pm$ 2.0	2
	200	39.8 $\pm$ 39.5	2	28.6 $\pm$ 0.8	2*

*Significantly different from Ringer's, $P < 0.05$.

Lever-press responses were reinforced either by electrical stimulation of the medial forebrain bundle on a continuous schedule or by presentations of sweetened milk on an aperiodic schedule (average, once every 2 min). All drugs were dissolved in 10 μl of Ringer's solution and injected in the lateral ventricle either 20 min after the start of the 40-min brain-stimulation reward test or immediately before the 72-min food reward test. N, number of rats.

Fig. 1 Intraventricular self-administration of opiate peptides and morphine. *a*, 10 μg per injection; *b*, 1 μg per injection. Curves show mean number of self-injections, cumulated at successive 6-h intervals over the entire 66-h test, of different groups of rats. For further details see Table 1. ■, Tyr-Gly-Gly-Phe-Leu; ×, morphine SO_4 (0.5 μg); ●, Tyr-Gly-Gly-Phe-Met; ▲, Tyr-Gly-Gly-Phe; ○, Ringer's.



Eight rats with electrodes in or just lateral to the pontine central grey received self-stimulation training until their 1-h response rates had stabilised (range, 1,200–5,000 responses per h). The central grey region was selected for initial testing because electrical stimulation of this site induces profound analgesia as well as high-rate self-stimulation¹⁶. Naloxone hydrochloride (0.1–10 mg kg^{-1} , subcutaneously) was administered at weekly intervals immediately before the behavioural test. As predicted, dose-related decreases in the self-stimulation rate were obtained in virtually every case (Fig. 2). Because the central grey region contains high concentrations of noradrenaline¹⁷, a substance previously shown to be involved in self-stimulation¹⁸, the same rats were further tested with different doses of the noradrenaline synthesis inhibitor, diethylthiocarbamate. Again,

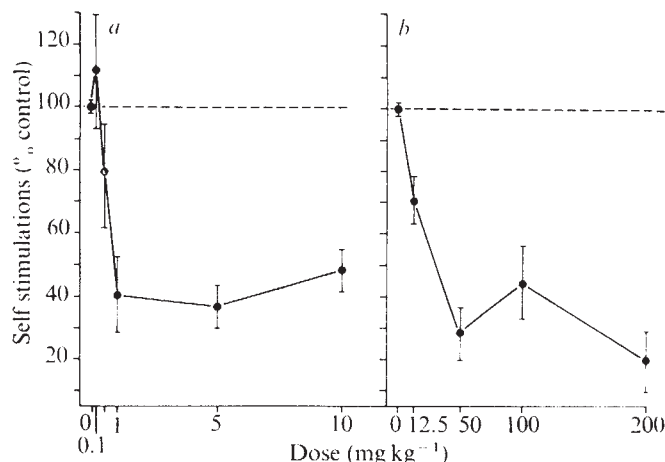


Fig. 2 Dose-related suppression of central grey self-stimulation following subcutaneous administration of the opiate antagonist naloxone (a) or the dopamine-β-hydroxylase inhibitor diethylthiocarbamate (b). Each data point is the mean of 3–8 observations. Bars indicate standard errors.

response rates were suppressed in a dose-related fashion (Fig. 2). These results suggest that central grey self-stimulation may depend on the activation of both nor-adrenaline-containing and enkephalin-containing neurones; indeed, since all of the enkephalin-rich self-stimulation sites referred to above are also rich in catecholamines¹⁷, it is possible that the reward process may generally be regulated by the interaction of noradrenaline, dopamine, and enkephalin systems. Studies on intravenous self-administration of opiate drugs similarly suggest that catecholamine mechanisms are involved in opiate reinforcement^{19,20}.

Diverse, but complementary, reward functions can also be recognised at the behavioural level. One of these corresponds to a state of incentive, and is the process by which behaviour in pursuit of a goal is motivated and steered; this 'drive-inducing' reward function may be mediated by catecholamines²¹. The second corresponds to a state of satisfaction or well being, and is the process by which an episode of behaviour is ended following the attainment (and consumption) of the goal; this 'drive-reducing' reward function may be mediated by an opiate peptide such as leucine-enkephalin.

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Selective vulnerability of neurones in organic dementia

THERE are many causes of the clinical state dementia. Excluding those patients with tumours, infection or vascular disease, there remains a large group¹ whose brains are atrophied and contain an excess of senile morphological change (senile plaques, neurofibrillary degeneration and granulovacuolar change) in the cortex. When this condition occurs before the age of 65 years it is known as presenile dementia or Alzheimer's disease; after this age it has been called senile dementia. Since there is no good reason on pathological grounds² to maintain this distinction, we use the term Alzheimer's disease irrespective of the patient's age. Together with Parkinson's disease and Huntington's chorea, Alzheimer's disease is one of a group of disorders that Gowers (see ref. 2) called abiotrophies, in which nerve cell loss appears to occur in characteristic patterns (that is, substantia nigra, basal ganglia and cortex, respectively). The cause of this cellular death remains unknown. Morphological assessment of possible nerve cell loss² or glial cell increase³ has been difficult, particularly in Alzheimer's disease. We have sought therefore to complement histological findings by measuring appropriate biochemical constituents of brain as indices of total neural cell numbers. Whole temporal lobes have been homogenised and analysed and the results expressed per lobe. The results⁴ suggest that whilst the total number of glial cells is relatively unchanged, there is a loss of approximately half the total nerve cell population. This estimate is based on comparison with age-matched controls, and so the selective neuronal loss is probably a disease-related phenomenon. These findings conform with conclusions drawn from morphological data⁵.

Evidence of selective changes in the number or state of specific types of neurone has been obtained by measuring the concentration of putative neurotransmitters and their biosynthetic enzymes. Thus there is selective reduction in the concentrations of dopamine⁶ in the substantia nigra and GABA⁷ in the striatum in patients with Parkinson's disease and Huntington's chorea respectively, suggesting a defect in dopaminergic or GABA-containing neurones. Choline acetyltransferase (CAT), and glutamic acid decarboxylase (GAD) are believed to be markers of cholinergic and GABA-containing neurones, respectively⁸. In Huntington's chorea GAD activity is selectively depleted only in morphologically affected regions^{9,10}. There are indications, however, that in Alzheimer's disease, the activity of CAT is reduced (compare groups 4 and 5, Table 1) and that this parallels increasing senile morphological change⁸, which in turn correlates with dementia score¹¹.

It is unclear whether or not our earlier finding of reduced cortical GAD activity in Alzheimer's disease (group 5, Table 1) accurately reflects the pre-terminal state of GABA-containing neurones⁸. This is because most demented patients die from severe bronchopneumonia⁸ and probably also have a defective respiratory reflex¹² with reduced cerebral blood flow terminally¹³. This is important, for GAD activity and the concentration of a soluble acidic brain protein (neuronin S-6) seem to decline both in baboon brain with reduced cerebral blood flow¹⁴ and in human brains from non-demented patients either dying of bronchopneumonia (compare GAD activity in groups 3 and 4, Table 1) or with evidence of more severe terminal cerebral hypoxia⁸. Furthermore, the concentration of neuronin S-6 is markedly depleted in cortex from cases of Alzheimer's disease^{8,15}. There are indications¹⁶ that this decrease in GAD activity is because of denaturation at acidic pH values. Our findings, and those of McGeer and McGeer¹⁰ on the effects of terminal coma, demonstrate the importance of making allowance for the agonal state.

In an attempt to resolve these difficulties we have examined cortical biopsies removed at craniotomy (group 2, Table 1). Most of these were cases of Alzheimer's disease, and the mean CAT activity is reduced by over 50% while GAD activity is

Table 1 Choline acetyltransferase (CAT) and glutamate decarboxylase (GAD) activities in human post-operation and post-mortem neocortical specimens

Brain specimen and group no.	CAT activity (units per min)	GAD activity (units per min)	Total protein (mg per g wet weight)
Post-operation specimens			
(1) Control tissue*	66.80 ± 26.96 (10)	1333 ± 467 (8)	81.2 ± 17.5 (10)
(2) Alzheimer's disease and related disorders†	27.52 ± 15.38 (6) ^a	1393 ± 329 (5)	75.7 ± 17.6 (6)
Post-mortem tissue‡			
(3) Controls dying suddenly	62.78 ± 14.75 (12)	1090 ± 460 (17)	74.6 ± 14.9 (17)
(4) Controls dying of bronchopneumonia	71.83 ± 16.42 (7)	690 ± 182 (8) ^c	75.1 ± 18.4 (7)
(5) Alzheimer's disease dying of bronchopneumonia	36.62 ± 10.65 (6) ^b	295 ± 89 (9) ^d	75.1 ± 10.4 (8)

CAT, GAD and protein were measured as previously described⁸. Data are mean ± s.d.; numbers in brackets are number of cases; enzyme units are pmol acetylcholine or GABA formed per mg protein. *—†Identify significant differences between closely matched groups. CAT activity: ^agroup 2 compared with 1, $P < 0.01$; ^bgroup 5 compared with 4, $P < 0.001$. GAD activity: ^cgroup 4 compared with 3, $P < 0.05$; ^dgroup 5 compared with 4, $P < 0.001$.

*Normal neocortex removed at surgery for tumours; mean age 42 ± 20 (range 10–67). For these specimens neither CAT nor GAD are age-dependent which contrasts with data for post-mortem samples from cases aged 5–50 (ref. 23).

†Neocortex from 2 biopsy confirmed cases of Alzheimer's disease and 2 probable cases; an adolescent with cerebral atrophy and some symptoms of schizophrenia ('dementia praecox') and a case of Parkinson's disease with a sister with pre-senile dementia; mean age 49 ± 18 (range 18–65).

‡Calculated from previously published data for neocortex from cases matched with respect to the interval between death and autopsy. The ages for groups 3, 4 and 5 are 72 ± 13 (range 50–100), 66 ± 16 (range 37–84) and 85 ± 5 (range 79–97). For these specimens neither CAT nor GAD activities are age-dependent⁸.

unaffected. For the four Alzheimer cases alone CAT activity (24.10 ± 18.63 units) is reduced ($P < 0.02$) by a mean of 64% while GAD activity (1298 ± 205 units) is unchanged. The results for the biopsy samples are unique for this is the first time that CAT activity in diseased human brain has been shown to be reduced with GAD activity remaining unaltered. Comparable results have been reported recently¹⁷ for the density of putative acetylcholine and GABA-receptor binding sites in striatum in Huntington's chorea.

The depletion in CAT activity that we have detected is of particular interest because the anticholinergic glycolate esters, of which the muscarinic cholinergic marker quinuclidinyl benzilate¹⁷ is a prominent example, can cause complete amnesia¹⁸. Furthermore, despite inconsistencies in the literature¹⁹, at least one pharmacological study²⁰ on humans indicates that the cholinergic system is involved in age-related memory degeneration. In order to provide a rational basis of treatment other tentative markers of the state and number of specific types of neurones are being measured. Preliminary results are encouraging for in one case (a 63-yr-old patient that probably had Alzheimer's disease) CAT activity in the biopsy was reduced by 67% and the high affinity uptake of choline was found to be reduced by 66% (the control uptake data ± s.d. expressed as c.p.m. per mg protein per c.p.m. per ml medium¹⁴ being 0.0097 ± 0.0029 , $N = 7$). In contrast, GAD activity and guanylate and adenylate cyclase activities¹⁶ (the latter being potential indices of the post-synapse) and β-alanine independent GABA uptake¹⁴ were all within 1.5 s.d. of the appropriate mean control value.

When the data for the biopsy and autopsy samples are considered together they suggest that at least the presynaptic cholinergic system is selectively affected in Alzheimer's disease. This perhaps offers the possibility of treatment with a centrally acting anticholinesterase for at present only about 14% of cases of organic dementia are treatable²¹. This therapeutic approach may not be entirely successful, however, as there are indications that other aspects of brain metabolism may be decreased, which may interact with the presynaptic cholinergic system²².

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Note added in proof: Data published recently^{24–27} substantiate our contention that the pre-synaptic part of the cholinergic system might be selectively affected in Alzheimer's disease.

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Catechol oestrogens inhibit oestrogen elicited accumulation of hypothalamic cyclic AMP suggesting role as endogenous anti-oestrogens

THE catechol oestrogens, including 2-hydroxyoestradiol and 2-hydroxyoestrone, are recognised as major metabolic products of oestrogen¹. Catechol oestrogens are present in both pituitary and hypothalamus in concentrations at least ten-fold higher than the parent oestrogens². In addition, the

synthesis of catechol oestrogens by rat hypothalamic tissue *in vitro* has also been reported^{2,3}. Catechol oestrogens compete with 17- β -oestradiol (E_2) for soluble oestrogen receptors of the pituitary and hypothalamus⁴, suggesting a role for these compounds in the control of gonadotrophin secretion within the pituitary-hypothalamic axis. We have investigated the effects of 2-hydroxyoestradiol on the oestrogen-elicited accumulation of cyclic AMP in intact hypothalami *in vitro*, and we report here that 2-hydroxyoestradiol antagonises the increase in cyclic AMP elicited in hypothalami of immature female rats by both diethylstilboestrol (DES) and E_2 , suggesting that 2-hydroxyoestradiol may function as an endogenous anti-oestrogen.

Confirming previous reports^{5,6,12,13}, both DES and E_2 significantly stimulated the accumulation of cyclic AMP in incubated rat hypothalami. Preincubation of hypothalami with equimolar (20 μ M) concentrations of 2-hydroxyoestradiol significantly inhibited the DES and E_2 stimulated accumulation of cyclic AMP by 57% and 68%, respectively (Table 1). At 20 μ M, 2-hydroxyoestradiol alone did not significantly stimulate the accumulation of cyclic AMP. In contrast to inhibitory effects of 2-hydroxyoestradiol, its *O*-methylated metabolite (2-methoxyoestradiol) was inactive at concentrations up to 50 μ M. Previous studies⁵ have shown oestrone and oestriol to have no significant stimulatory effects on the accumulation of cyclic AMP. Equimolar (20 μ M) concentrations of either oestrone or oestriol inhibited DES-elicited accumulation of cyclic AMP only marginally. The anti-oestrogen clomiphene citrate (20 μ M) was previously shown to antagonise⁵ (by 50%) the effects of DES (20 μ M); this finding was confirmed in the present study (41% inhibition, see Table 1).

The ability of oestrogenic compounds to stimulate the formation of cyclic AMP in the isolated hypothalamic

preparation seems to parallel the oestrogenic potency of these compounds both *in vivo* and *in vitro*^{5,6}. In addition, the oestrogen-elicited increases in cyclic AMP are antagonised by the anti-oestrogen clomiphene and are not observed with physiologically inactive oestrogen enantiomers such as 17- α -oestradiol. Furthermore, both clomiphene and 2-hydroxyoestradiol are ineffective in antagonising the effects of DES if added 40 min after the oestrogen, and both α - and β -adrenergic antagonists are effective inhibitors^{6,12}. These observations suggested that an initial binding to hypothalamic oestrogen receptors is a prerequisite for DES- or E_2 -elicited accumulation of cyclic AMP and the stimulatory effects of oestrogens on cyclic AMP formation result from an oestrogen-receptor dependent release catecholamines rather than a direct activation of an oestrogen-receptor linked adenylate cyclase⁶.

Our data clearly demonstrate that the catechol oestrogen 2-hydroxyoestradiol, like clomiphene, antagonises the effects of both DES and E_2 on generation of cyclic AMP in the hypothalamus, whereas weakly oestrogenic compounds such as oestriol and oestrone are ineffective as either agonists^{5,6} or antagonists. The observation that 2-methoxyoestradiol does not antagonise the effects of DES *in vitro* indicates that *O*-methylation may serve as a major route of inactivation for these compounds as this pathway proceeds readily both *in vitro*¹⁴ and *in vivo*¹⁵.

The presence of catechol oestrogens in brain and various endocrine tissues in concentrations that exceed those of the parent compounds² suggests that these catechols could function as endogenous anti-oestrogens, and thus may regulate the actions of oestrogens *in situ*. Reports that administration of 2-hydroxyoestrone to ovariectomised, oestrogen-primed female rats¹⁶ or immature male rats¹⁷

Table 1 Inhibition of oestrogen-elicited accumulation of hypothalamic cyclic AMP by 2-hydroxyoestradiol

Series I	Additions	Cyclic AMP (pmol per mg protein)	Inhibition (%)
	None	8.9 $\pm$ 0.9 (8)	
	DES (20 μ M)	25.5 $\pm$ 2.4 (7)	
	2-hydroxyoestradiol (20 μ M)	9.0 $\pm$ 2.6 (3)	
	DES (20 μ M) + 2-hydroxyoestradiol (20 μ M)	15.9 $\pm$ 2.2 (6)	57*
	DES (20 μ M) + 2-methoxyoestradiol (50 μ M)	25.5 $\pm$ 1.5 (3)	0
	17- β -oestradiol (20 μ M)	12.0 $\pm$ 0.5 (5)	
	17- β -oestradiol (20 μ M) + 2-hydroxyoestradiol (20 μ M)	9.9 $\pm$ 0.9 (4)	68†
Series II	Additions	Cyclic AMP (pmol per mg protein)	Inhibition (%)
	None	6.3 $\pm$ 0.6 (7)	
	DES (20 μ M)	18.4 $\pm$ 0.5 (6)	
	DES (20 μ M) + clomiphene citrate (20 μ M)	13.4 $\pm$ 1.4 (5)	41§
	DES (20 μ M) + 2-hydroxyoestradiol (20 μ M)‡	16.8 $\pm$ 1.5 (6)	14
	DES (20 μ M) + oestrone (20 μ M)	16.3 $\pm$ 1.8 (7)	17
	DES (20 μ M) + oestriol (20 μ M)	18.6 $\pm$ 1.4 (6)	0

Female Sprague-Dawley rats (Taconic Farms), aged 26–28 d were used. Animals were killed by decapitation and hypothalami removed and dissected^{5,6}. Hypothalami from groups of 10 rats were incubated at 37 °C in 20 ml Krebs–Ringer bicarbonate buffer with glucose (KRBG)⁷ and gassed with 95% O_2 –5% CO_2 . After 45 min hypothalami were washed once with an equal volume of KRBG and transferred to individual 30-ml beakers containing 10 ml of KRBG plus test agents. Steroids were dissolved in 75% ethanol and added in a volume of 20 μ l. Hypothalami were pre-incubated with catechol oestrogen (or clomiphene) for 10 min before addition of oestrogen. Hypothalami receiving oestrogen alone received an equal volume of 75% ethanol 10 min before addition of oestrogen. After addition of oestrogenic compounds incubations were continued for an additional 70 min and terminated by homogenisation of hypothalami in 1 ml of 5% trichloroacetic acid. Recovery of cyclic AMP was monitored by adding 0.1 pmol of ³H-cyclic AMP (22 Ci mmol⁻¹). After centrifugation, 300 μ l of supernatant was withdrawn, 20 μ l of 1 N HCl was added, and the trichloroacetic acid supernatant was extracted four times with three volumes of diethyl ether. Residual ether was removed by heating at 56 °C for 3 min. Samples were frozen on dry ice and lyophilised. Cyclic AMP was determined by the protein binding technique of Gilman⁸ as modified⁹. Protein was determined by the Miller¹⁰ modification of the Lowry¹¹ technique. Inhibition (as%) of oestrogen effects by putative antagonists was calculated by the formula

$$\left[1 - \left(\frac{(\text{oestrogen} + \text{antagonist}) - \text{basal}}{(\text{oestrogen}) - \text{basal}} \right)\right] \times 100$$

Steroids, radioisotopes and reagents were from sources described before^{5,6}. Values are mean $\pm$ s.e.m.

* $P < 0.02$ Student two-tailed t test.

†Not significantly different from no additions.

‡2-Hydroxyoestradiol added 40 min after DES.

§ $P < 0.01$, Student two-tailed t test.

produces marked elevations in plasma luteinising hormone are also consistent with an anti-oestrogen action for the catechol oestrogens.

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Prenatal exposure of human fetuses to synthetic progestin and oestrogen affects personality

THE offspring of mothers given steroid hormones for the maintenance of at-risk pregnancy provide a human population equivalent to experimental animals for investigating early hormone effects on behaviour and personality¹. Although patients showing the genetically induced clinical endocrine syndromes² associated with diminished or augmented prenatal hormone levels have been used as an alternative source of subjects, they are less useful, as results attributed to the altered hormone environment can also be attributed to genes linked to the primary source of the endocrine dysfunction. Investigations using offspring of hormone-supported pregnancies are equivalent to the animal studies because in both cases the source of hormone intervention is exogenous. We report here that prenatal exposure to synthetic progestins and oestrogens affects later personality in humans.

Hormones administered to support at-risk pregnancy include both natural and synthetic progestins and oestrogens. Many of the synthetic progestins are similar chemically and in action to androgen^{3,4,5} and in some cases masculinise the genitalia of exposed females. Only three samples of prenatally hormone-exposed individuals have been studied previously^{3,6,7}. Unfortunately, all three of these studies suffer from design problems including lack of controls or inappropriate control groups, small sample size, and incomplete treatment information. Although individuals born of pregnancies treated with steroid hormones provide the best population with which to study the effects of the prenatal hormone environment on human behaviour, the paucity of such studies is understandable due to the difficulty of identifying and locating subjects

whose mothers received hormonal intervention during pregnancy and appropriate control groups. This investigation was designed to overcome the problems of the previous studies and to expand their findings.

Six hundred records from two Los Angeles clinics and a private medical practice were reviewed. Thirty-four families were identified in which at least one pregnancy had been treated with synthetic progestin alone or in combination with oestrogen and at least one pregnancy had not been treated with these hormones. Of the 600 cases reviewed, only two children exhibited congenital abnormalities; namely a heart defect and missing fingers and toes. All available offspring of every family were evaluated for inclusion in the study. To participate, offspring of the treated and untreated pregnancies had to conform to the following criteria—the same parentage within families; at least 5 yr of age at time of study; and complete doctor and hospital records on pregnancy and delivery. Thirty-six physicians and 50 hospitals across the United States furnished the pregnancy and delivery records for the unexposed siblings and additional information on the treated pregnancies. The criteria for qualification as a treated pregnancy included a dosage minimum of 450 mg progestin (range 478–9,890); duration of at least 4 weeks (range 4–36); and treatment beginning in the first trimester of pregnancy. The final sample comprised 42 hormone-exposed subjects aged 5–17 yr (15 males and 27 females) and 42 unexposed siblings (6–18 yr; 18 males and 24 females). Birth order was balanced between exposed and unexposed subjects.

It was thought that a group of subjects could be found whose mothers were treated with a similar and consistent hormone regime during pregnancy, but review of medical records showed that treatment schedules, dosages and hormone combinations varied considerably not only among doctors but from patient to patient. Physicians frequently administered several different progestins and/or oestrogens to a single woman over the course of pregnancy. The most generally administered hormone preparations were Colprosterone, Norlutin, Delalutin, Deluteval, Provera, Provest and diethylstilbestrol. This necessitated the *post hoc* strategy of segregating subjects into two groups based on treatment regime. Group placement was defined by the ratio of progestin to oestrogen administered to the mothers of exposed subjects. The 26 members of the progestin group (P_o) were exposed to a mean ratio of 548 mg progestin to 1 mg oestrogen, when they were exposed to both hormones. Mothers of 17 subjects from this group received no oestrogen at all. Mean progestin intake per mother was 2,994 mg (range 525–9,890). The 16 subjects in the oestrogen group (O_o) were exposed to more oestrogen than progestin. The mean ratio of progestin to oestrogen was 1:4 and the mean total dose of oestrogen per subject was 6,119 mg (range 3,500–13,905). Mean ages for exposed subjects in the P_o and O_o groups were 12.46 and 12.06 yr respectively; mean ages of unexposed siblings for both the P_o and O_o groups were 11.8 yr at time of testing. Subjects were given the age-appropriate Wechsler intelligence scale and Cattell personality questionnaire by testers with no knowledge of the purposes of the study and the treatment category of the child. These instruments were selected for their comparability of scores across age groups.

Analysis of covariance was applied to IQ and personality scores to eliminate possible confounding influences such as heredity—family environment and prenatal and perinatal complications. The covariate of unexposed sibling score acted as a statistical control for heredity and family environment and prenatal and perinatal complications scores (severity and number of pregnancy and delivery problems) acted as controls for the at-risk nature of the treated pregnancies. Statistical application of these covariates to

the scores of subjects in the exposed groups adjusted them so that they could be considered as having been generated from subjects obtained through pre-experimental random assignment to groups. This adjustment provided a *post hoc* statistical technique yielding a basis for the assumption of equality of groups before the application of treatment. Covariance analysis was also used on difference scores generated by subtracting the score of the unexposed sibling from that of the exposed subject within families—only prenatal and perinatal complications scores were applied as covariates.

Prenatal hormone exposure significantly affected subjects' responses to the personality measure. Significant differences between the P_o and O_p treatment groups were found on five personality factors: P_o group subjects were significantly more independent, sensitive, individualistic, self-assured and self-sufficient than the exposed offspring of the O_p group, while the O_p group subjects were more group-oriented and group-dependent (Table 1).

Table 1 Progesterin (P_o) and oestrogen (O_p) group means for five personality factors

	Ind.	I	J	O	Q_2
P_o	7.30	6.75	7.82	2.54	6.70
O_p	5.13	5.28	4.04	5.07	3.11

Mean scores of P_o and O_p groups on five factors from the Cattell personality questionnaires (Early School Personality Questionnaire, Children's Personality Questionnaire, High School Personality Questionnaire, and 16 Personality Factors) which yielded statistically significant between group differences ($P < 0.05$). The factors are bipolar dimensions with the high-score pole versus (vs) the low-score pole for each as follows: Ind. (independent vs subdued); I (sensitive vs tough-minded); J (individualistic vs group-oriented); O (insecure vs self-assured); and Q_2 (self-sufficient vs group-dependent). The possible range in which an individual's score can fall is 1–9 with the norm by age and sex set at 5.

Similar results, consistent with the between groups analysis, were obtained when exposed subjects were compared with their unexposed siblings within families (Table 2). The exposed subjects of the P_o group were significantly more independent, individualistic, self-assured and self-sufficient than their siblings. The subjects of the O_p treatment group were significantly less individualistic, and therefore more group-oriented than their siblings and less self-sufficient and therefore more group-dependent. An integration of these findings results in the characterisation of the P_o group subjects as 'inner' or 'self' directed and those exposed to the O_p regime as 'outer' or 'other' directed in relation both to the other treatment group as well as their siblings.

The P_o group profile corresponds well to the findings of Ehrhardt & Money³. The mothers of their progesterin exposed subjects reported high levels of self-assertive independence and self-reliance and correspondingly low frequencies of dependency and demand for succorance when describing their offspring.

In contrast to the earlier report³, we found no significant differences on any of the scores generated from the Wechsler IQ tests. The full scale IQ for the exposed subjects of the P_o group was 121.08 with 120.12 for their siblings. The O_p group mean was 122.25 in contrast to 120.19 for their siblings. It seems that exposure to synthetic progesterin alone or in combination with oestrogen has no effect on IQ, neither augmenting nor diminishing scores, when exposed subjects are compared to the base line provided by the family. The scores of both exposed and unexposed subjects, however, were 20 points higher than the norm, similar to the results obtained in the study of 10 genitally-masculinised girls³. The consistently elevated

Table 2 Group mean difference scores on five personality factors

	Ind.	I	J	O	Q_2
P_o	+1.19*	+0.37	+1.39*	-1.30*	+2.93*
O_p	-0.50	-0.65	-1.40*	-0.06	-3.67*

Mean difference scores of P_o and O_p groups on five factors from the Cattell personality questionnaires (see Table 1 for explanation of factors). A difference score is derived by subtracting the score of the unexposed sibling from the score of the exposed sibling. A positive score indicates that the exposed subject received a higher score than the unexposed sibling.

*Significant difference between exposed and unexposed siblings ($P < 0.05$).

scores probably reflect the socioeconomic status (SES) of these families who undertook this expensive and time-consuming treatment to support at-risk pregnancy (76%, or 26 families fell into the highest SES categories, I and II, of the Hollingshead scale and no family was from the lowest category, V). As would be expected, the best predictor of the later IQ scores of exposed subjects was the IQ of their siblings ($r=0.46$).

Finally, Cattell has provided a configuration of six personality factors which research identified as having a significant relationship to school achievement and success⁴. The P_o group subjects exhibited high scores on three of these factors (individualistic, self-assured, and self-sufficient), whereas, the O_p groups were significantly low on two (group-dependent and group-oriented). Since there were no significant differences between the treatment groups or between exposed and unexposed siblings on the IQ tests, the groups are in effect matched for IQ. The prediction of higher school achievement in this sample of P_o regime exposed subjects is consistent with the results from the longitudinal study of prenatally progesterone exposed subjects which demonstrated significantly higher school achievement from childhood to late adolescence⁶.

Our finding demonstrating a long term effect of early exposure to steroid hormones supports the investigations with animals and extends them solidly into the human realm. The data have shown that exposure to synthetic progesterin and oestrogen during the foetal period significantly affects performance on a test designed to assess personality. Of course, to demonstrate conclusively a long term effect on personality in humans contingent upon prenatal exposure to various hormones, longitudinal studies of these and other individuals whose gestations were treated are necessary. The wealth of data related to early hormone influences on behaviour in animals⁷ strongly suggests, however, that exposure to hormones of exogenous origin in humans should have some long term effects on such dimensions as personality and/or temperament⁸.

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Synthesis and processing of ribosomal RNA by the uterus of the ovariectomised adult rat during early oestrogen action

THE rate of RNA metabolism in the uterus of the immature or ovariectomised adult rat is stimulated after the administration of oestrogen^{1,2}. Although there is uncertainty concerning the nature of the RNA species synthesised by the uterus during the first hour of oestrogen action², it is generally agreed that by 2 h after the injection of the hormone there is a significant increase in the synthesis of ribosomal RNA³⁻⁵. Furthermore, Luck and Hamilton^{5,6} have suggested, on the basis of sucrose gradient analysis of labelled uterine RNA, that the rate or efficiency of processing of precursor ribosomal RNA (pre-rRNA) is also increased by the hormone. We have investigated the effect of oestrogen on the metabolism of rRNA in the uterus of the ovariectomised adult rat and now report the results of gel electrophoretic analyses of uterine RNA, doubly labelled *in vivo* with (³H-methyl)methionine and ¹⁴C-uridine. In the atrophied uterus of the ovariectomised adult rat the rate of conversion of pre-rRNA to the definitive rRNA products was slow, and it increased with time after hormone administration reaching a maximal rate after 4 h.

stimulation. After 3 and 4 h of hormone treatment the ³H in the tissue was increased 26% and 45%, respectively. These observations are consistent with previous reports^{5,7} and illustrate the usefulness of methionine as a precursor for labelling methylated RNAs synthesised during hormone action.

Oestrogen stimulated the uptake of ¹⁴C-uridine by the uterus 48% after 1 h of treatment, 8% after 2 h, 12% after 3 h, and 17% after 4 h (Table 1). The percentage incorporation of ¹⁴C-uridine into whole organ RNA was not significantly increased by hormone action. Oestrogen also increased the specific activity of the isolated ¹⁴C-labelled RNA 75% after 1 h of treatment, 52% after 2 h, 28% after 3 h, and 40% after 4 h. These results are somewhat at variance with a previous report⁶, in which, however, 50 μ Ci of ¹⁴C-uridine was injected into each animal, rather than 5 μ Ci. The present results compare favourably with those reported by Munns and Katzman⁷. The incorporation ratio of (³H-methyl)methionine to ¹⁴C-uridine increased with time after hormone administration, following an initial lag period of about 1 h (Table 1). This finding is in good agreement with previous observations³⁻⁵ that an increasing percentage of the newly synthesised RNA formed by the uterus during the first few hours following oestrogen stimulation is methylated.

The absorbance and radioactivity profiles of the gel electrophoretic analyses of the samples of labelled RNA isolated in the experiment described in Table 1 are shown in

Table 1 Time course for the effect of oestrogen on the ratio of RNA to DNA and the uptake and incorporation of (³H-methyl)methionine and ¹⁴C-uridine into RNA in the uterus of the ovariectomised rat

Hormone treatment time (h)	RNA-DNA ratio	Total tissue radioactivity (d.p.m. per mg DNA)		Radioactivity incorporated into whole-organ RNA (%)		Specific activity of isolated RNA (d.p.m. per mg)	
		³ H	¹⁴ C	³ H	¹⁴ C	³ H	¹⁴ C
0	0.34	532,300	10,200	3.9	36	9,200	6,700 (1.4)*
1	0.36	535,000	15,100	4.9	32	15,800	11,700 (1.4)
2	0.39	538,900	11,000	5.2	39	18,400	10,200 (1.8)
3	0.42	672,800	11,400	5.5	40	26,300	8,600 (3.1)
4	0.45	770,700	11,900	6.3	40	44,400	9,400 (4.7)

RNA was isolated from the combined uteri of four ovariectomised adult rats that received 20 μ g of 17- β -oestradiol in 0.2 ml of 1,2-propanediol (or the solvent alone), intraperitoneally (i.p.), at the times indicated before killing. All animals received 250 μ Ci L-(17- β -methyl)methionine (11 Ci mmol⁻¹) and 5 μ Ci ¹⁴C-uridine (55 mCi mmol⁻¹) in 0.4 ml of water i.p. 45 min before killing. The method for isolating samples of undegraded RNA from uteri of ovariectomised adult rats was as described previously, except for the following two modifications. A second extraction of the interfacial material was performed by gently homogenising it with 4 ml of aqueous phase and stirring at 40 °C for 20 min. The phenol solutions contained an equal volume of chloroform-4% isoamylalcohol. The final yield of isolated, purified RNA averaged 67%. RNA and DNA determinations were carried out as before, as were the measurements of radioactivity incorporated⁶.

*Ratio of incorporation of methionine to uridine.

Table 1 shows the effect of oestrogen, as a function of time, on the uptake and incorporation of (³H-methyl)methionine and ¹⁴C-uridine into uterine RNA as well as the specific activity of the RNA isolated from uteri of ovariectomised animals that received the radioactive precursors simultaneously 45 min before death. The ratios of RNA to DNA in the various uterine homogenates revealed that oestrogen increased the concentration of RNA in the organ (compare with ref. 5). The total incorporation of (methyl-³H)methionine into whole-organ RNA during the 45 min labelling period was increased 134% by hormone treatment for 4 h. Oestrogen also markedly increased the specific activity of the ³H-labelled RNA isolated. It is important to emphasise that the data presented (Table 1) demonstrate that oestrogen has little or no stimulatory effect on the whole-organ uptake of methionine during the first 2 h of

Fig. 1. The absorbance profiles of all the RNA samples analysed were essentially the same. The ¹⁴C profiles (not shown) revealed that uridine had been incorporated into RNA very heterogeneous in size in both the control and hormone-stimulated uterus. No differences in the shapes of the ¹⁴C profiles could be attributed to oestrogen. The ³H profiles, however, reflected differences in the relative amounts of the various classes of labelled precursor, intermediate and mature rRNA species present in the uterus at various times after the injection of the hormone. Incorporation of (³H-methyl)methionine into 45S, 32S, 28S, 21S and 18S rRNAs took place during the 45-min labelling period in both control and hormone-treated animals. In the unstimulated uterus the percentage of ³H found in the precursor 45S and 32S rRNAs totalled 49% of the label incorporated into all rRNA species. But, as the time of the

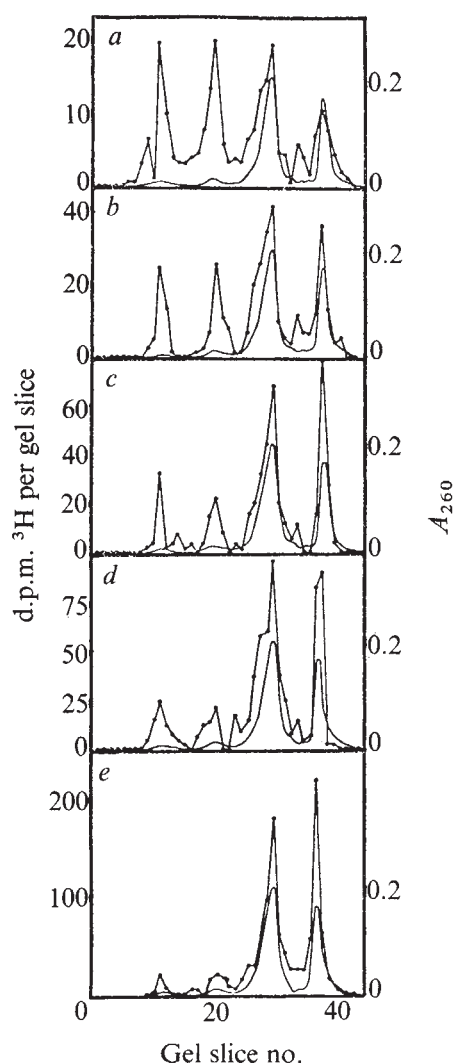


Fig. 1 Absorbance and radioactivity profiles of isolated, uterine RNA, labelled *in vivo* with (^3H -methyl)methionine, as a function of time after administration of oestrogen. Experimental details are as in Table 1. Rats received a, no hormone, or oestrogen for b, 1 h; c, 2 h; d, 3 h; or e, 4 h. (●—●), ^3H ; —, A_{260} . Samples of doubly-labelled RNA, of exactly 0.5 A_{260} , were fractionated as described by Caston and Jones⁸ on 1.8% polyacrylamide-1% agarose gels prepared in quartz tubes (90 $\times$ 5 mm inside diameter). Electrophoresis was for 2½ h at 5 mA per gel. Unlabelled 28S and 18S rRNAs were isolated from rat liver ribosomes and initially used as markers, as was 45S pre-rRNA isolated from nuclei of Novikoff ascites cells. Gels were scanned at 260 nm in a Gilford spectrophotometer and sliced into 2-mm slices. Each slice was placed in a counting vial and 0.1 ml of 30% ammonium hydroxide added to hydrolyse the RNA. After standing overnight at room temperature 1 ml of NCS tissue solubiliser (Amersham/Searle) was added to each vial and all vials were shaken for 1 h. Scintillation fluid, 10 ml, (5 g PPO and 0.1 g POPOP per litre of toluene) was added to each vial and the radioactivity determined to an error of 5% or less in a Beckman liquid scintillation spectrometer. A ^3H counting efficiency of 44% was obtained.

hormone treatment increased this percentage decreased until 4 h after oestrogen administration only 12% of the total amount of methyl-labelled rRNA formed was present in the 45S and 32S fractions (Table 2). Longer periods of stimulation by the hormone did not significantly alter this distribution of the label among the 45S, 32S, 28S and 18S fractions. Labelled precursor was available to the uterine cells during the entire 45-min labelling period as evidenced by the presence of (^3H -methyl)methionine in the organ at

the time of killing of the animals. These data demonstrate that during the first 4 h of oestrogen action in the uterus of the ovariectomised adult rat the amount of new methylated 28S and 18S rRNA formed from the methylated 45S precursor per unit time is increased nearly fourfold.

We have routinely observed a methyl-labelled fraction slightly larger than 45S in uterine RNA samples isolated from the control animals which was not present following oestrogen stimulation (see Fig. 1a). Interestingly, Galibert *et al.*⁹ have reported that there are three distinct components in the 45S pre-rRNA fraction of L-cells separable by electrophoresis through 1.7% polyacrylamide gels. Whether this heterogeneity represents a population of

Table 2 Effect of oestrogen on the incorporation of (^3H -methyl)-methionine into ribosomal RNAs by the rat uterus

Hormone treatment time (h)	45S (%)	32S (%)	28S (%)	18S (%)
0	21	28	32	19
1	19	18	39	24
2	12	11	47	30
3	10	9	51	30
4	5	7	55	33

The percentages in this table are calculated from the radioactivity profiles shown in Fig. 1. The label in the 45S fraction was computed by summing the d.p.m. in gel slices 5–15; the 32S fraction by summing gel slices 16–22; the 28S fraction, gel slices 23–32; and the 18S fraction, gel slices 33–41.

slightly different initial precursor molecules or early processing intermediates remains to be elucidated.

Post-transcriptional mechanisms for regulating rRNA synthesis have recently been proposed in phytohaemagglutinin-stimulated lymphocytes^{10,11}, during compensatory renal hypertrophy¹², in a mutant of BHK cells^{13,14} and in liver¹⁵. It is not yet clear, however, whether the accelerated rate of processing of pre-rRNA to 28S and 18S rRNAs in the oestrogen-stimulated uterus is due to more efficient methylation and cleavage, a more efficient processing pathway, or is determined by increased rates of rRNA synthesis¹⁶.

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Thioredoxin and glutathione regulate photosynthesis in chloroplasts

Two recently found chloroplast proteins act in the ferredoxin-linked regulation of enzymes of photosynthetic carbon dioxide assimilation^{1,2}. We report here the identification of one of these proteins, earlier termed assimilation regulatory protein b (ARP_b), as thioredoxin—a protein functional in the synthesis of DNA and previously not known in chloroplasts³⁻⁵. The other protein, assimilation regulatory protein a (ARP_a), is a new enzyme that catalyses the reduction of thioredoxin by reduced ferredoxin. We have renamed this protein 'ferredoxin-thioredoxin reductase'. In addition to identifying these proteins found earlier, we present evidence for a regulatory function of glutathione in chloroplasts. These findings permit for the first time the formulation of a light-dependent mechanism for protein-mediated enzyme regulation. In this mechanism, key regulatory enzymes are activated (reduced) photochemically via the ferredoxin-thioredoxin system and are deactivated (oxidised) in the dark via oxidised glutathione. The evidence indicates that photoreduced ferredoxin, in the presence of ferredoxin-thioredoxin reductase, controls the level of reduced thioredoxin required for enzyme activation. Glutathione reductase and glutathione peroxidase seem to control the level of oxidised glutathione available for enzyme deactivation.

Fructose 1,6-bisphosphatase of chloroplasts was assayed by measuring the release of inorganic phosphate (P_i) from the carbon-1 of fructose 1,6-bisphosphate^{1,6}. Thioredoxin from *Escherichia coli* could fully replace native ARP_b in the light-induced activation of chloroplast fructose 1,6-bis-phosphatase (Table 1). In this system, enzyme activation required ferredoxin, reduced photochemically with chloroplast fragments, and ARP_a in addition to either ARP_b or *E. coli* thioredoxin. ARP_b and *E. coli* thioredoxin were also found to be equally effective when their capacity for phosphatase activation was tested in the presence of the non-physiological sulphhydryl reagent dithiothreitol and in the absence of reduced ferredoxin and ARP_a (refs 1, 6; Table 2).

The dithiothreitol assay was used previously to demonstrate that ARP_b exists in the major types of living cells³. ARP_b was found not only in photosynthetic plant and bacterial cells but

Table 2 Equivalence of chloroplast ARP_b and *E. coli* thioredoxin in the activation of chloroplast fructose 1,6-bis-phosphatase by dithiothreitol

Treatment	nmol P _i released per min
Chloroplast ARP _b , 12 µg	50
<i>E. coli</i> thioredoxin, 10 µg	47
Minus ARP _b or thioredoxin	7

The reaction was carried out in air at 25 °C in test tubes containing, in 0.5 ml, fructose 1,6-bis-phosphatase, 9 µg; ARP_b or thioredoxin as indicated, and the following (µmol): Tris-HCl buffer (pH 7.9), 50; MgCl₂, 0.5; dithiothreitol, 2.5; sodium fructose 1,6-bis-phosphate, 3. The reaction was started by adding fructose 1,6-bis-phosphate and was continued for 30 min. The reaction was stopped by adding 2 ml of a mixture of the Fiske-SubbaRow reagents used to analyse P_i (see Table 1).

also in their non-photosynthetic counterparts and in animal cells. In view of the ability of *E. coli* thioredoxin to replace ARP_b in activation of the chloroplast fructose bisphosphatase, it seems likely that the active component found earlier in the different types of cells was thioredoxin. The known wide distribution of thioredoxin is in accord with that conclusion⁷.

The observation that *E. coli* thioredoxin is active in assays specific for ARP_b suggests that ARP_b might substitute for thioredoxin in the synthesis of DNA. This was confirmed by the finding that chloroplast ARP_b showed thioredoxin activity in the *E. coli* ribonucleotide reductase assay system. Chloroplast ARP_b replaced native thioredoxin to a low but significant extent in the enzyme-mediated reduction of ribonucleotides to their deoxy derivative. The low activity of chloroplast ARP_b in this assay is attributed to its weak affinity for the enzymes of DNA synthesis in *E. coli*.

Our results show unforeseen similarities between two proteins that were discovered in very different lines of research—thioredoxin was found in studies on the synthesis of DNA in *E. coli* and ARP_b was found in studies on the light-dependent regulation of enzymes in chloroplasts. It now seems that these two proteins are functionally similar. We therefore consider a revision of our original nomenclature timely and designate ARP_b 'chloroplast thioredoxin'. By analogy, the protein earlier called ARP_a is, on the basis of its ability to catalyse a thioredoxin-dependent oxidation of reduced ferredoxin (Table 3), designated 'ferredoxin-thioredoxin reductase'. We found no indication that chloroplasts contain an *E. coli*-type NADP-thioredoxin reductase⁸ that links NADPH to the activation of fructose 1,6-bis-phosphatase.

The ferredoxin-thioredoxin system constitutes a new mechanism which, in chloroplasts, links light to the regulation of certain enzymes of carbon dioxide assimilation. An important aspect of this or any other system of enzyme regulation is the

Table 1 Interchangeability of chloroplast ARP_b and *E. coli* thioredoxin in the activation of chloroplast fructose 1,6-bis-phosphatase by photoreduced ferredoxin

Treatment	nmol P _i released per min Chloroplast ARP _b	<i>E. coli</i> thioredoxin
Light, complete	12	10
Light, minus ARP _b or thioredoxin	2	2
Light, minus ferredoxin	4	2
Light, minus ARP _a	1	1
Light, minus fructose 1,6-bisphosphatase	0	1
Dark, complete	2	1

The reaction was carried out at 20 °C in Warburg vessels containing (in the side arm) 6 µmol of sodium fructose 1,6-bis-phosphate and (in the main compartment) 17 µg of fructose 1,6-bis-phosphatase dimer²⁶, spinach chloroplast fragments (P₇₀) equivalent to 0.1 mg chlorophyll¹⁰, 0.12 mg *Spirulina maxima* ferredoxin²⁷, 70 µg ARP_a (in an aliquot containing 3 µmol 2-mercaptoethanol), and the following (µmol): Tris-HCl buffer (pH 7.9), 100; neutralised reduced glutathione, 5; sodium ascorbate, 10; 2,6-dichlorophenolindophenol, 0.1; MgSO₄, 1.0. ARP_b, 25 µg, or *E. coli* thioredoxin, 20 µg, were added as indicated. Final volume, 1.5 ml. After equilibration with nitrogen for 6 min, vessels were incubated for 5 min in the light (20,000 lx) or in the dark. The reaction was started by adding fructose 1,6-bis-phosphate from the side arm and was continued for 30 min under illumination or in the dark. The reaction was stopped by adding 0.5 ml of 10% trichloroacetic acid. After centrifuging off the precipitate, the P_i released was estimated by a modified Fiske-SubbaRow procedure¹⁰. ARP_a and ARP_b were purified as before^{1,6}. ARP_a was then further purified by DEAE-cellulose and hydroxyapatite column chromatography (details to be published elsewhere).

Table 3 Identification of chloroplast ARP_a as ferredoxin-thioredoxin reductase

Component added to oxidise photoreduced ferredoxin	nmol ferredoxin oxidised per min
None	0.0
ARP _a	0.6
Chloroplast thioredoxin	0.9
ARP _a + chloroplast thioredoxin	12.0

The reaction mixture contained, in 1.2 ml, *Spirulina maxima* ferredoxin, 0.18 mg; chloroplast fragments¹⁰ (P₇₀) equivalent to 5 µg chlorophyll; and the following (µmol): Tris-HCl buffer (pH 7.9), 100; MgSO₄, 1; sodium ascorbate, 10; and 2,6-dichlorophenolindophenol (DPIP), 0.1. ARP_a, 230 µg (in an aliquot containing 6 µmol 2-mercaptoethanol) was added as indicated. Samples were gassed with argon for 5 min and transferred anaerobically to a glass cuvette of 2-mm light path. After fully reducing the ferredoxin photochemically by illumination with red light (Corning 2-58 filter), the light was turned off, and the rate of the oxidation of ferredoxin was followed in the dark. The extent of oxidation of ferredoxin was measured by increase in absorbance at 420 nm with a modified Beckman Model DU monochromator²⁸.

Table 4 Deactivation of chloroplast fructose 1,6-bisphosphatase by oxidised glutathione

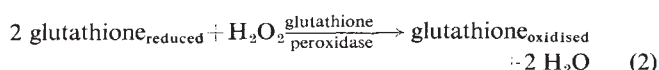
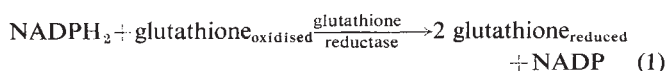
Reductant used for activation	% of control activity after adding glutathione
Reduced ferredoxin*	53
Dithiothreitol†	5

*The reaction mixture was as in Table 1 except that 25 μmol oxidised glutathione were added to the second side arm and 104 μg ferredoxin-thioredoxin reductase (ARPA) (plus 3 μmol 2-mercaptoethanol) were used. Vessels were equilibrated with nitrogen for 6 min and incubated 10 min in the light (20,000 lx). The reaction was started by adding fructose 1,6-bisphosphate from the first side arm. After 10 min under illumination, the lights were extinguished, oxidised glutathione (or water) was added from the second side arm, and the reaction was continued for 20 min in the dark. The reaction was stopped and P_i was analysed as in Table 1. 15 nmol of P_i were released per min in the control treatment.

†Fructose 1,6-bisphosphatase was activated (and deactivated) before assay. Fructose 1,6-bisphosphatase, 17 μg , was incubated in 0.1 ml of a mixture containing 0.1 M Tris-HCl buffer (pH 7.9), 10 mM dithiothreitol, and 25 μg chloroplast thioredoxin (ARPB). After 10 min, 2.5 μmol of oxidised glutathione (or water) was added. After a further 20 min, the enzyme was then injected into 0.9 ml of the assay mixture containing 1.3 units of glucose 6-phosphate dehydrogenase, 4.2 units of glucose 6-phosphate isomerase, and the following (μmol): Tris-HCl buffer (pH 7.9), 40; MgSO_4 , 1; fructose 1,6-bisphosphate, 6; and NADP, 1.5. Fructose 1,6-bisphosphatase activity was followed by measuring the reduction of NADP (increase in absorbance at 340 nm) with a Cary model 14 spectrophotometer. Temperature, 22 °C. 29 nmol of P_i were released per min in the control treatment.

way in which the component enzymes are converted from an active to a less active (deactivated) state. Until now, no deactivation mechanism was known for the ferredoxin-thioredoxin system. As seen in Table 4, glutathione—a sulphhydryl-disulphide component of most if not all living cells⁹—functioned in that capacity. The oxidised form of glutathione deactivated the fructose bis-phosphatase that had been activated previously by thioredoxin reduced either photochemically with the ferredoxin system or non-enzymically with dithiothreitol. Under the same conditions, reduced glutathione had no effect.

The effectiveness of oxidised glutathione in deactivation suggests that *in vivo* a defined ratio of oxidised to reduced glutathione gauges the activity of enzymes, such as fructose bisphosphatase, in response to changing conditions in the chloroplast. This ratio, in turn, may be determined by two enzymes present in the soluble fraction of chloroplasts¹⁰, glutathione reductase¹¹ (equation 1) and glutathione peroxidase¹² (equation 2).



The specific activity in μmol substrate converted per mg chlorophyll per h was 9 for glutathione reductase¹³ and 12 for glutathione peroxidase¹⁴. Because of its relatively high activity, it seems possible that glutathione peroxidase could, in addition to its regulatory role described here, function together with glutathione reductase in removal of hydrogen peroxide produced in chloroplasts. Accordingly, glutathione peroxidase would replace catalase, which seems to exist mainly outside rather than inside chloroplasts^{15,16}. Our finding of 1 mM glutathione¹⁷ in chloroplasts is consistent with such a function for glutathione peroxidase.

Although the physiological agent for enzyme deactivation seems to be oxidised glutathione, other oxidants were also effective in this reaction. Sodium tetrathionate ($\text{Na}_2\text{S}_4\text{O}_6$) and the oxidised form of ascorbic acid (dehydroascorbic acid) showed deactivation effects similar to those reported in Table 4 for oxidised glutathione. In view of the high concentration of

ascorbic acid in chloroplasts^{18,19}, these results indicate that dehydroascorbic acid, produced from ascorbic acid through oxidation, could possibly be involved in the control of enzyme activity.

Considering the wide distribution of ferredoxin and thioredoxin, a comment on the possible application of the ferredoxin-thioredoxin system to other organisms seems appropriate. It is possible that a mechanism of the type described could be used for the regulation of cellular processes in other organisms. Furthermore, because many cells are enzymically fitted to reduce thioredoxin independently of ferredoxin, by way of NADP, the presence of ferredoxin is not prerequisite to the operation of such a system. For example, a mechanism of the NADP-thioredoxin type could account for the thioredoxin-mediated reduction of proteins (for which the reported reduction of insulin provides a model²⁰) or for modification of proteins through the formation of a stable thioredoxin-protein complex (as found for a specific DNA polymerase in phage-infected *E. coli* cells²¹).

The present results provide evidence for a new mechanism whereby specific enzymes are activated by reduction (via thioredoxin) and deactivated by oxidation (via oxidised glutathione). In chloroplasts this 'oxidation-reduction' mechanism acts in conjunction with the ion-mediated and effector-mediated mechanisms discussed previously^{1,22} which link enzyme regulation to the absorption and utilisation of light. In the light, electrons from chlorophyll are transferred to ferredoxin and then, via a specific reductase enzyme, to thioredoxin (Fig. 1). Reduced thioredoxin, in turn, reduces and thereby

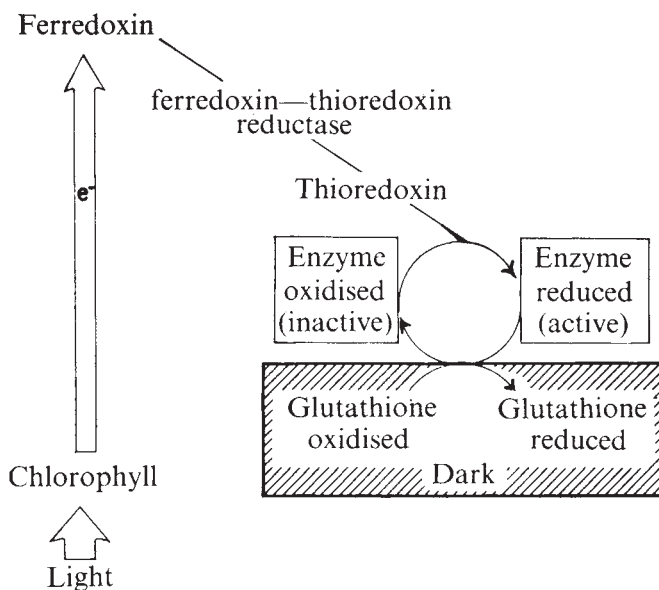


Fig. 1 A mechanism for the light-dependent regulation of enzymes in chloroplasts. The component³⁰⁻³² before the indicated soluble ferredoxin that accepts electrons from photo-activated chlorophyll is not shown.

activates certain regulatory enzymes of chloroplasts (fructose 1,6-bisphosphatase, sedoheptulose 1,7-bisphosphatase²³, and perhaps NADP-glyceraldehyde 3-phosphate dehydrogenase and phosphoribulokinase^{1,24}). Our evidence indicates that, at least in the case of NADP-glyceraldehyde 3-phosphate dehydrogenase, reduction (activation) is accompanied by the formation of a stable thioredoxin-enzyme complex.

In the dark, the activated (reduced) enzymes are deactivated (oxidised) by an oxidant such as oxidised glutathione. Figure 2 shows that, in the presence of hydrogen peroxide, glutathione peroxidase could act in the dark to convert reduced glutathione to the oxidised form that is functional in deactivation. It is likely that limited enzyme deactivation would occur also in the light in response to transient changes in the chloroplasts.

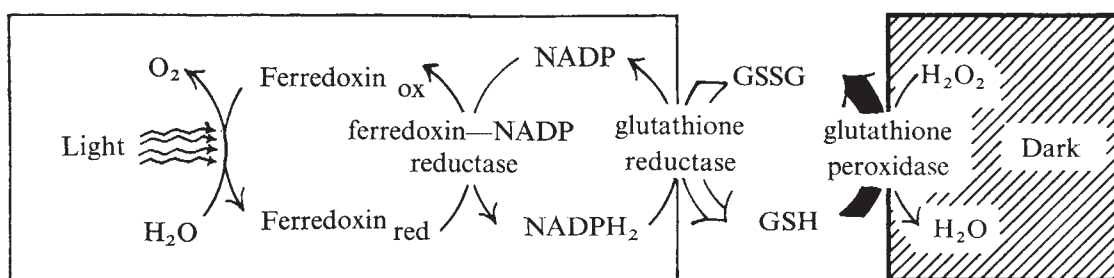


Fig. 2 A mechanism for controlling the ratio of oxidised (GSSG) to reduced (GSH) glutathione in chloroplasts.

The high concentration of reduced NADP prevailing in those conditions²⁵, however, would tend to impede such deactivation by converting the oxidised glutathione to its reduced form with the chloroplast enzyme glutathione reductase.

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Was photoresolution of amino acids the origin of optical activity in life?

SINCE Pasteur showed that the amino acid asparagine in plants was purely one optical isomer, constant interest has centred around the question of optical activity in life. The quantities of D-amino acids in the biosphere are practically negligible and none has ever been found in any enzyme or other protein, whereas the abundance of L-amino acids is well known. Many of the molecules important to living processes can be syn-

thesised¹ in conditions which may have prevailed on a primitive planet and it is easier to suggest a prebiotic synthesis of a racemate than to explain an enantiomeric selection. Whereas many workers claim the clue might be found in various stereo-selective mechanisms, likely to have operated already at the stage of the 'chemical evolution'^{2–5}, no example of the selection of a biologically crucial compound by means of circularly polarised light has been previously reported. Apart from an effect due to an enantiomorphous relationship between matter and antimatter^{6–8}, this is the only mechanism with selection capacity in a large system (the Earth)⁹. We report here that tartaric acid, α-alanine and glutamic acid partially resolve under circularly polarised irradiation. The L-amino acid dominance in the biosphere can by analogy, be explained by a selection caused by circularly polarised interstellar ultraviolet radiation.

Pairs of identical racemic aqueous solutions were simultaneously subjected to left and right circularly polarised light (quartz collimators/Glan prisms/Fresnel rhombs) from a Xe (450 W quartz, Osram) lamp, λ > 200 nm (aqueous infrared filter) and compared afterwards with respect to circular dichroism difference at the wavelengths of photolysis (Jasco J-41 dichrometer). Results are given in Table 1 and Fig. 1.

Fig. 1 Typical circular dichroism spectra ($\times 10^6$) recorded on a, racemic tartaric acid and b, glutamic acid after irradiation with right and left circularly polarised light. c, Recorded ΔCD deflections ($\times 10^6$) at 210 nm in glutamic acid at different irradiation times (different symbols for different experiments).

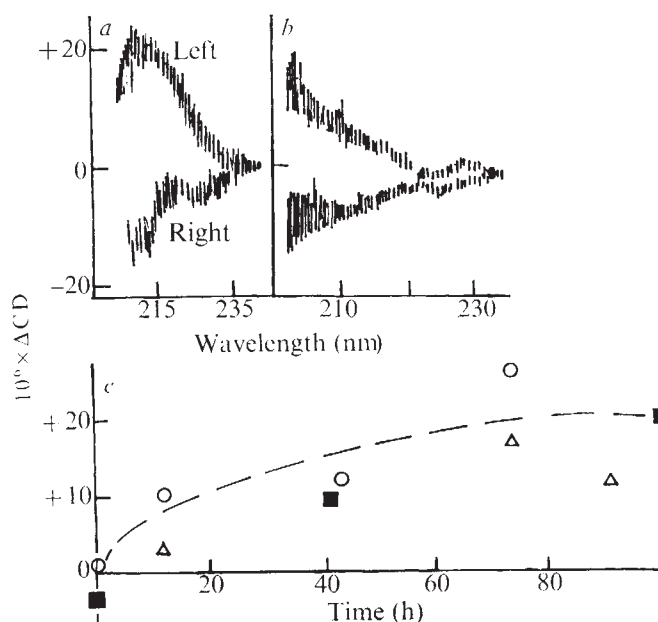


Table 1 Observed and calculated selection

Specimen	Wavelength (nm)	$ \Delta\epsilon/\epsilon $ of pure enantiomer	Irradiation time (h)	Conversion (inversion plus decomposition) (%)	$10^6 \times \Delta CD$ normalised to $A_0 = 1$, between left and right circularly irradiated solutions (absorbance units)	Enrichment yield in each sample (%)
DL-tartaric acid, 1 cm, 5×10^{-3} M in H_2O	250 230 220 215($\Delta\epsilon_{max}$)	0.002 0.020 0.020 0.020	30	> 20	42 ± 5	0.11* (0.8)†
DL-alanine, 1 cm, 10×10^{-3} M in H_2O	210–230 203($\Delta\epsilon_{max}$)	(0.010) 0.007				
DL-glutamic acid, 1 cm, 10×10^{-3} M in H_2O	230 225 210 202($\Delta\epsilon_{max}$)	(0.011) 0.008 0.008 0.007				
	210	0.008	504	52	36 ± 5	0.22* (0.35)†

* Defined as $100 \times (CD_{obs}/CD_{pure\ enantiomer}) \approx 50 \Delta CD/(\Delta\epsilon/\epsilon)$.

† Theoretically possible figures in parentheses, obtained as $100 \times (CD_{theor}/\Delta\epsilon\ C_l) \approx 40 (\Delta\epsilon/\epsilon)^2 A/Cl = 40 \Delta\epsilon/\epsilon$.

Due to uncertain lamp intensity in the active range (200–230 nm) relevant quantum yields could not be estimated.

It is realistic to assume that the activation is achieved either by photodecomposition or by photoinversion leading to excess of the enantiomer that has positive circular dichroism at the photolytical wavelength, when right circularly polarised light is used and vice versa. Some amino acids may undergo photo-deamination to yield cinnamic acids, where the unsaturated character can cause obscuring absorption preventing complete photolytic yield. When optically active tartaric acid and aliphatic amino acids were subjected to non-polarised ultra-violet light ($\lambda > 200$ nm) absorptive products were generally not observed and the rate of loss of optical activity indicated a simultaneous photoracemisation. In the case of pure photodecomposition the maximum absolute activity is expected when 63% of the material is decomposed, by a circular dichroism deflection¹⁰

$$|CD| = 0.37 \times (\Delta\epsilon/\epsilon)^2 A_0 \quad (1)$$

($\Delta\epsilon = \epsilon_1 - \epsilon_r$ and ϵ are molar, $M^{-1} \text{ cm}^{-1}$ circular dichroism and absorption coefficients, A_0 the initial absorbance. As was shown elegantly by Kagan¹¹ 100% purity can, however, be obtained at the end of the reaction. For photoracemisation a steady state

$$|CD| = 0.5(\Delta\epsilon/\epsilon)^2 A_0 \quad (2)$$

should be reached. In both cases it is presumed that only the excited molecule undergoes reaction (and that it does so due to the dichroic transition), that is, that no radical attacks or sensitising effects occur. By the quadratic appearance of the dissymmetry factor $\Delta\epsilon/\epsilon$ the possibilities of experimental detection are restricted to molecules with bands having $|\Delta\epsilon/\epsilon| > 3 \times 10^{-3}$ due to the resolution of present day instruments, which is $\pm 3 \times 10^{-6}$ absorbance units at unit absorbance. That the observed optical activity is due to optical selection among the starting materials and not to any optically active secondary product is indicated by the complete extinction of optical activity when the pure isomers are irradiated. The final proof was obtained by the observation of a constant line-shape of the CD-spectrum during the decay period.

Finally, it should be pointed out that a spectral requirement $\lambda > 200 \pm 30$ nm on the incident radiation is in excellent agreement with the current opinion of the composite ultraviolet absorption of the primitive atmosphere (due mainly to water vapour and carbon dioxide)¹². The L-amino acids (except

the cyclic imino-acid proline) are thus selected by this type of light (when right circularly polarised), while for instance nucleic acids should require $\lambda > 260$ nm for an effective yield (according to the circular dichroism spectrum with wings of both signs). We are also studying amino acid complexes with evolutionarily interesting metals and where magnetic dipole allowed d-d transitions (for example Cr(III)) can be expected to strongly enhance the photoresolution efficiency.

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reviews

Aquaculture methods

C. M. Yonge

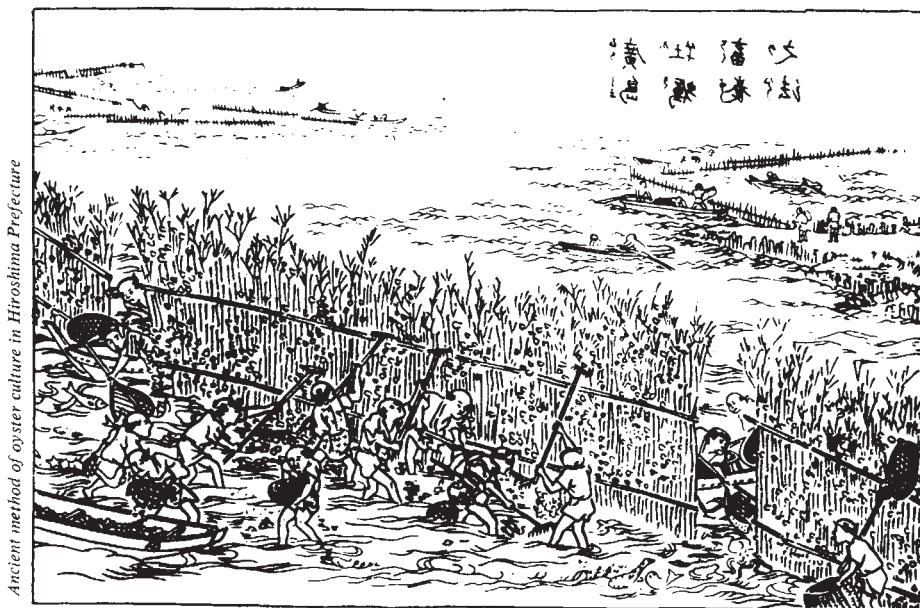
Farming Marine Organisms Low in the Food Chain. Farming Cupped Oysters of the Genus Crassostrea. Farming the Flat Oyster of the Genus Ostrea. Farming Marine Fishes and Shrimps. (Developments in Aquaculture and Fisheries Science, Vols 1-4.) By P. Korringa. (Elsevier: Amsterdam, Oxford and New York, 1976.) \$30.95; Dfl.80 each volume.

AT this time of increasing competition for already over-exploited stocks of fish, any increase in marine productivity by aquaculture demands attention. No one is better qualified to write about this than Professor P. Korringa, Director, Netherlands Institute for Fishery Investigations. Responsible for restoration of the Dutch oyster beds following shell disease in the 1930s, he must since have visited every significant area of marine cultivation. This reviewer has memories of visits in his company to areas of oyster and mussel cultivation in France, Spain and Italy.

His descriptions of culture methods are based on actual procedures within selected aquacultural enterprises. For each he supplies a biological, geographical, oceanographic and even legal background—thus sheltered, pollution-free waters and security of tenure are all involved. Farming procedures include control of predators, parasites and disease. Economic problems including marketing are finally assessed.

Description of culture methods begins with that of the red seaweed 'Nori' (*Porphyra*) in Japan and concludes with that of rainbow trout and salmon in Norway. Cultivation of bivalve molluscs is inevitably the major theme; mussels (*Mytilus* spp.) clams and, above all, species of the flat oyster, *Ostrea*, are covered together with those of the ecologically more tolerant cupped oyster, *Crassostrea*, which are everywhere replacing them.

Mussels, strangely neglected in North America and Japan, are reared on stakes in the long established 'bouchot' system in France, by mass transplantation in the Netherlands, and with impressive productivity in the sheltered



Ancient method of oyster culture in Hiroshima Prefecture

'rias' in north-west Spain, where settlement and growth are on ropes suspended from moored pontoons or catamarans.

The great French oyster industry was initially based on studies of the surviving remnants of Roman culture methods in the Mediterranean. Large scale cultivation, between tide marks—first of *Ostrea edulis*, then of the Portuguese *Crassostrea angulata* and now of the ubiquitous Japanese, *C. gigas*—was established; and its methods have now been widely copied throughout the world. The highly successful suspended culture method was initiated in Japan some fifty years ago, at first to offset restrictions caused by limited intertidal space.

All these methods involve collection of naturally produced spat in areas of relatively still water and subsequent growth in water rich in phytoplankton. Only in hatcheries (about which Dr Korringa has surprisingly little to say), where oysters are induced to spawn and the larvae and spat fed on reared algal species, can full culture be established.

With fish, only the cultivation of herbivores—here described for mullets (*Mugil*) in Israel and in 'valli' round the mouth of the Po in the Adriatic, and for milk fish (*Chanos*) long culti-

vated in Indonesia—represents economic production of animal protein. Japanese cultivation of the carnivorous yellow-tail (*Seriola quinqueradiata*) and red sea bream (*Chrysophrys major*)—with that of the no less carnivorous penaeid prawns (*Penaeus japonicus*)—involves conversion, with much accompanying labour, of lower into higher or more acceptable forms of animal protein. The result is an expensive luxury product.

Conditions are somewhat different with eel culture in Japan—supplying such a demand that elvers are imported from so far afield as Europe—and salmonid rearing in Norway and Scotland. These have the advantage of supplying an insatiable demand at a lower cost than that involved in collection of these fish in Nature.

These admirably produced and illustrated books are a pleasure to review. They will be of the greatest value to aquaculturists; nothing is described that has not been personally inspected and judged in the light of Dr Korringa's uniquely wide experience. □

Sir Maurice Yonge, formerly Professor of Zoology at Bristol and then Glasgow, is now Honorary Fellow in Zoology at the University of Edinburgh, UK.

Molecular genetics handbook

Handbook of Genetics. Vol. 5: Molecular Genetics. Edited by R. C. King. Pp.xi+667. (Plenum: New York and London, 1976.) \$51.

THE fifth volume of the *Handbook of Genetics* in many ways belies its title. The book is in four sections devoted to the molecular organisation of chromosomes (six chapters), gene transcripts (five chapters), chloroplasts and mitochondria (five chapters), and a single chapter in a section called mutant enzymes. Only the chapters in the section on chloroplasts and mitochondria seem truly to combine both a molecular and genetical approach and deserve the title molecular genetics.

Particularly disappointing is the *pot pourri* section on molecular organisation of chromosomes, which manages to present a somewhat incoherent treatment of the subject in spite of excellent reviews of chromosomal protein structure and function, and of organisation and size of replicons. Individual chapters on repeated DNA in eukaryotes, cytological localisation of repeated DNAs, chromosomal proteins, organisation of genetic material in the macronucleus of hypotrichous ciliates, histones of sperm, and organisation and size of replicons, do not easily relate to each other and the section as a whole presents no overall picture of chromosome organisation. The chapter on repeated DNA in eukaryotes, by limiting itself to molecular renaturation studies, manages to ignore much interesting and relevant data provided by sequencing, restriction site mapping and electron microscopy of repeated DNAs.

The chapter on chromosomal proteins, although admirable in many ways, contains no reference to the unit of chromatin (nucleosome) and models which have been proposed for its structure. Such an omission can hardly be of the authors own making since it seems evident from the reference list that this chapter was complete by the end of 1973 (the only 1974 paper quoted is by the authors themselves) and the same seems to hold for the majority of chapters in this section; one exception being the chapter on macronuclear DNA of hypotrichs, which has references to 1976. Judging by the latest references in chapters of other sections they were completed towards the end of 1974 or beginning of 1975, although the chapter on mutant enzymes has no references after 1972. It is a pity that

the relatively long lag between writing and publishing the chapters in section one has reduced its effectiveness as a contemporary review of a rapidly changing area of investigation.

The other sections are more successful. Section two, gene transcripts, has chapters on nuclear RNA, RNA transcription and ribosomal protein assembly in *Drosophila melanogaster*, cytoplasmic mRNA, tRNAs, and eukaryotic ribosomes; whereas section three, chloroplasts and mitochondria, has chapters on chloroplast DNA (physical and genetic studies), chloroplast ribosomes, nucleocytoplasmic interaction in *Acetabularia*, mitochondrial DNA, and mitochondrial ribosomes. The chapters on cytological localisation of repeated DNAs, histones of sperm, transfer RNAs, and mutant enzymes are little more than exhaustive catalogues of the structure, location or primary sequence of particular macromolecules.

Chemical relaxation

Relaxation Kinetics. By C. F. Bernasconi. Pp. xi+288. (Academic: New York and London, 1976.) \$29.50; £20.95.

THERE can be no getting away from it—a large proportion of chemical reactions are fast. Generations of students may have obtained their grounding in chemical kinetics with reactions whose half-lives lie conveniently in the time range of minutes or hours, but the fact is that such reactions are the exception rather than the rule. One has only to recall the particular premium placed on the time factor in many biologically and industrially important reactions to appreciate the significance of our being able to measure the kinetics of rapid reactions.

It is not possible to apply classical kinetic techniques to many of these reactions but over the past fifteen years or so a variety of methods have been developed which have made most of them susceptible to study. All of these methods involve electronic equipment and the prospect of having to build one's own apparatus, together with the fact that a rather different approach is generally required in the analysis of the data, has tended to scare away all but the most physically-minded chemists. Times are, however, changing and it is thanks to the increasing availability of suitable commercial equipment and monographs such as Dr Bernasconi's that more and more chemists are reporting rate constants and

In spite of some omissions which might have been included in a book with this title—for instance, considerable space is devoted to histones but no mention is made of histone genes, and similarly there is no mention of tRNA genes although all the known tRNA sequences are tabulated—there is a great deal of worthwhile and well presented material. As a reference book in certain areas, it should prove valuable.

The book has an author and subject index. But the subject index has innumerable deficiencies, and I pick one example to illustrate this point. The only reference to "globin" contains an interesting cross-reference "see Immunoglobulin". There is, however, no entry under "Immunoglobulin"; but under "Immunoglobulin" there is a reference to "chain of rabbit reticulocyte"! **Peter Ford**

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activation parameters where in the past they would have used some such phrase as "too fast to measure".

The first two-thirds of Dr Bernasconi's book deals with the general theory of chemical relaxation and is largely given over to the problem of relating the relaxation time (or times) and the rate constants for different mechanistic schemes. Also included are a couple of chapters (out of ten) on the topic of relaxation amplitudes. The remainder of the book is concerned with the various experimental methods.

The author says that he has attempted to write a comprehensive treatment of the subject in the language of the organic or physical-organic chemist and in this I think he has on the whole been successful. Certainly, the mathematics should not overtax any mechanistically-minded research chemist (contrary to the impression he might gain on casually leafing through it) and there are many useful hints and practical comments hidden (the correct word, unfortunately) throughout the book.

Where I would be a little less than fulsome in praising the book is for its comprehensiveness. Although his choice will certainly not be universally applauded, it seems to me that Dr Bernasconi has quite properly and freely exercised his author's discretion on the topics for inclusion. Presumably, this is the reason behind his implication (with which I would not agree) that this is not a book for the physical-inorganic chemist. **D. N. Hague**

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Sedum of North America

Sedum of North America, North of the Mexican Plateau. By Robert T. Clausen. Pp.742. (Cornell University: Ithaca and London, 1976.) £42.25.

Sedum is the largest genus of the family Crassulaceae and consists of about 300 species, most of them in the north temperate zone but some on subtropical and tropical mountains. They are succulent plants, usually low-growing and small-leaved, and on account of their general easiness of cultivation and propagation and their numerous small starry flowers they have long been esteemed as rock-garden plants. Their garden value led an Irish botanist, Robert Lloyd Praeger, to publish in 1921 an account of the genus *Sedum* as found in cultivation (*J. R. Hort. Soc.*, 46, 1-314) describing and illustrating 151 species, still a most useful work. A Swedish botanist, Harald Fröderström, published between 1930 and 1935 a survey of the genus as a whole in *Acta Horti Gothoburgensis*, distinguishing 327 species. Neither competes in bulk and wealth of detail with Robert T. Clausen's *Sedum of North America*, which devotes 742 pages to some 90 species, only 36 of them native. Unlike other workers on the genus, which he has studied intensively since 1935, Professor Clausen has been able to examine wild populations of almost every North American species and has supplemented his field surveys by experimental culture at Cornell University. Thus he has been able to separate environmental modifications from genetically fixed characters.

The result is this comprehensive monograph admirably illustrated with various sketch maps and with line drawings of all the species by Elfriede Abbe, who earlier provided those in Clausen's *Sedum of the Trans-Mexican Volcanic Belt* (1959). That work set out to be "an exposition of taxonomic methods", not simply a concise working account of 28 Mexican species, and provided a prolixity of detail irrelevant for almost all its users. In *Sedum of North America*, because the area covered is about 216 times larger (though with almost the same number of species), the same treatment has resulted in this huge and expensive work. It raises the question as to whether the printing (as distinct from the research and recording) of so many measurements in the diffuse paper-covering style of this book is justifiable nowadays, since the information relevant for most purposes could have been presented in a volume of less than half the size and half the price. It thus suffers from a defect produced by its major virtue. Having worked so long, so thor-

oughly and so successfully at understanding these plants which can indeed only be properly evaluated from living material studied both in the wild and under cultivation, Professor Clausen has evidently felt he must publish everything, an honest enough procedure but costly. Thus his account of *Sedum spathulifolium* occupies 35 pages; he nevertheless leaves somewhat uncertain Praeger's var. *purpureum*, of which he has seen no material, although this is still cultivated in England.

For anyone interested in the *Sedum* species of North America, their characters, population variation, nomenclature, distribution and identification, this work will be indispensable, which makes its high cost all the more regrettable. Likewise regrettable is the lack of attention

to page headings; instead of the verso heading throughout the book unnecessarily proclaiming that it deals with "Sedum of North America", it could more usefully have given the chapter heading, while the recto heading then named the species dealt with beneath, which is often not evident. One hopes that some day Professor Clausen will produce a convenient illustrated handbook to the genus *Sedum* in America as a whole, summarising concisely the results of his many years of painstaking enquiry.

W. T. Stearn

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Statistical mechanics of liquids

Theory of Simple Liquids. By J. Hansen and I. R. McDonald. Pp. xv+395. (Academic: London and New York, 1976.) £14; \$30.65.

RECENT years have shown an upsurge of interest in the statistical mechanics of liquids, which has been marked by a number of important advances. The advance has been continuous since 1957; the previous history of the subject had been a series of fundamental advances, with long gaps in between.

This book is a timely and balanced survey of recent developments, shows how they are related to the older work, and explains the relationships between the various theoretical approaches. The year 1957 showed that the task of evaluating the correct consequences of an assumed intermolecular interaction function was within the power of large computers and this has stimulated a great deal of further work. One good feature is a special chapter describing techniques of these computer calculations.

The days of analytical results in this field are, however, very far from over, and these approaches are described in chapters 4, 5 and 6. Basically what has happened is that the fundamental advances made by Kirkwood (the feasibility of an integral equation approach) and by Mayer (the diagram expansion technique) have been systematically followed up and improved. It has also been found possible to develop perturbation theories analogous to those of quantum mechanics and celestial mechanics. These three chapters provide a connected account of these developments, contain very little repetition and are a pleasure to read.

Some workers consider equilibrium statistical mechanics to be difficult

enough and fight shy of transport processes. A large amount of work has, however, been carried out, and is well summarised in chapters 7, 8 and 9, which the reviewer, largely an 'equilibrium man', found most helpful. In reading a published paper in this field it can be quite difficult to separate the formalism and the 'intelligent guess-work' or to see whether any difficulties of principle are being glossed over. These chapters will certainly be helpful to many workers, as will the account of generalised hydrodynamics, hard to find elsewhere except in original papers.

I was disappointed to see no mention of surface tension and related phenomena. One of the first definite results in liquid-state theory was Laplace's relation between surface tension and latent heat, and the subject was again opened up by the pioneer work of Kirkwood and Buff. Many of the published papers are, however, obscure and complicated; perhaps lack of space was the deciding factor. The final chapter, on phase transitions, seems to have suffered for a similar reason. The discussion of melting is as good as could have been hoped for in the present rather fragmentary state of knowledge but I would have liked to see a far more extensive account of the critical region. In particular, I would have liked to know the authors' own views on the following point. Why do scaling and renormalisation group methods provide good results despite the fact that they seem to use approximations just as drastic as those that vitiated older theories of phase transitions?

The book is well printed and produced but the price seems on the high side even at present levels. There is a valuable bibliography and a good index.

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Drama of environmental physiology

Environmental Physiology of Animals. Edited by J. Bligh, J. L. Cloudsley-Thompson and A. G. MacDonald. Pp. viii+456. (Blackwell Scientific: Oxford, 1976.) Cloth £18; paper £9.50.

THIS book is a multi-author text for degree-level students who have at least some knowledge of physiology and ecology. Its scope is very wide for, as the editors state in a postscript (p434) "Between a mutation and the resultant marginal benefit detectable in a population there lies an enormously complicated drama acted out by individual organisms in an environment which is certainly indifferently and often hostile. Environmental physiology is this drama, and is thus the central subject of biology. It embraces the intracellular molecular biology which accounts for the occurrence of mutations, as well as classical unicellular and multicellular morphology, physiology, biochemistry and ecology. It demands the ability to think at different levels of analysis—at those of molecules, of cells, of organisms and of populations, because organisms adapt in different ways at these different levels".

The text is divided into four main sections, and there is also an editorial introduction (part I) and postscript (part VI). Apart from the editorial contributions, the main text consists of a short section (part II) of two chapters on the origin of life and the evolution of the oceans and atmosphere. This is followed by a major section (part III) of seven chapters on adaptive mechanisms which have resulted from long-term evolutionary changes. It includes excellent reviews on ionic and osmotic regulation in aquatic animals, water balance and excretion, gas exchange, and reproduction. Part IV on acquired adaptations contains a well-coordinated and stimulating series of chapters on non-genetic adjustment to change in environmental conditions. This is followed by a section (part V) on immediate homeostatic or sensory responses. The latter contains chapters on sensory physiology, buoyancy, colour change, and temperature regulation.

A feature of this book is the contributions by the editors to coordinate the text so that contributory chapters can be placed clearly into the context of the framework of the book as a whole. It is a praiseworthy attempt to overcome some of the problems associated with multi-author texts, and some of the prefaces and introductory chapters to each section are quite outstanding. Chapter 11 introducing

the concept of acclimatory adaptation and chapter 16 on rapid responses of organisms to environmental change, are especially clear in introducing later chapters in each section. Other chapters are, however, less successful in this respect and are in places even slightly ludicrous in attempting to unite environmental concepts with those of physiology. After identification of the principal terrestrial environments in chapter 6, for example, the reader is informed (p102) that "The topography of the land and variations of its climate represent the same part in the make up of the environment, figuratively speaking, as morphology and physiology do in the life of an animal (12). Thus irrespective of climate, the mammalian fauna of desert, savanna, steppe, tundra and high plateaux have certain features in common". I was unable to fully understand either the precise meaning of the analogy attributed to Haveland (1926), or the logic of its relationship with the statement that follows it.

It was a little disappointing to find that there was no chapter on sense organs in the section on evolutionary aspects, and that no detailed consideration was given to topics such as acoustic interactions between bats and their prey in chapter 17, a topic which would surely have fitted in

well with the concepts underlying the theme of this book. It was also slightly surprising to find in the chapter on locomotion that there were no diagrams of how fishes, quadrupeds and flying animals actually conform to Newton's three Laws of Motion, despite the excellent prelude to chapter 8 stating these as underlying principles. Perhaps these were assumed to be well-known from other texts, but it would surely have been well-worth illustrating them in a comprehensive book designed for the undergraduate level.

The contributions overall are of very high quality, and are remarkably free of mistakes. The book has been well compiled and conscientiously edited so that, in general, individual chapters are well-balanced reviews which fit the stated theme of the book. The continuity is greatly aided by the editorial contributions, some of which are exceptionally stimulating. This book will be a welcome addition to the shelves of the physiologist and ecologist. It is enjoyable to read and is to be highly recommended for courses in environmental physiology at university level.

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Nematodes

The Organization of Nematodes. Edited by N. A. Croll. Pp. viii+439. (Academic: London and New York, 1976.) £15; \$32.75.

THIS multi-author volume contains fourteen chapters which describe various aspects of the structure, physiology and biochemistry of nematodes. The controversial topic of nematode taxonomy is discussed in the first chapter and is followed by chapters which review, to a greater or lesser extent, energy metabolism; the functional organisation of the head; the development and organisation of skeletal structures; sense organs and their secretions; nitrogen excretion, osmotic and ionic regulation; nematodes as models for ageing studies; respiratory physiology; hormones; neuromuscular physiology; the mechanics of feeding; behavioural coordination; the use of mutants to analyse the sensory system; and survival strategies.

The individual chapters of this book are of a high standard, and some are excellent. In a book with this title, however, one would expect complete coverage of all fields of the organisation of nematodes, but the alimentary system, with the notable exception of the oesophagus, and the reproductive systems, with the exception of the spicules and the egg-shell, are not included; yet these are major systems in nematodes.

The reviews are also not grouped in a logical manner. This may be deliberate policy to emphasise the individual nature of each chapter, but one would, for example, expect to find energy metabolism, respiratory physiology and nitrogenous excretion dealt with in adjacent chapters. There is also overlap of subject matter in several chapters. This is inevitable in a multi-author volume, but sense organs, for example, are described in chapters 3, 5 and 13, with no cross references. It is unfortunate that the authors of these particular chapters were apparently not given access to each other's manuscripts. Several chapters are comprehensive reviews of the subject, whereas some others deal with a smaller number of examples or topics in greater depth. Some chapters include important work which has not previously been published. It is a matter for regret that the plates, especially the electron micrographs, do not occupy more of the page, as certain details have been lost as a result.

This book is a significant contribution to the literature on nematodes, and will be extremely useful to those who teach and carry out research on these important animals. It will also be heavily used by students who wish to pursue, in depth, the various topics dealt with in the book.

D. L. Lee

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Recent scientific and technical books

Biological Sciences

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Applied Biological Sciences

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- DISEASES OF THE LIVER AND BILIARY TRACT: Standardization of Nomenclature, Diagnostic Criteria, and Diagnostic Methodology. (Fogarty International Center Proceedings No. 22.) (DHEW Publication No. (NIH) 76-725.) Pp.xiv+212. (Washington, DC: US Government Printing Office, 1976.) \$4.90.
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newly on the market

These descriptions are prepared by the staff of *Nature* on the basis of material provided by manufacturers. There is a Reader Enquiry Form on page xxi.

HPLC Column Injector System. Shandon Southern. The system comprises an optimised septum injector or an adaptor allowing the use of other injectors, a range of columns up to 8 mm bore, and a packing tube for slurry packing. These are designed as a unified system and combine the high efficiency of syringe injection with a standard of reproducibility normally associated with valve injection systems. Both analytical and small scale preparative chromatography are possible. **Circle No. 33 on Reader Enquiry Form**

High-Resolution Underwater Detection System. Marconi. A digital sonar navigation, positioning and detection aid for the location, tracking and recovery of underwater objects such as well-heads, pipelines and divers known as Marfix, this portable acoustic detection system employs a new method of sonar electronic scan. Marfix comprises three main units: a processor, a display and a portable transducer which can be mounted on most vessels to scan either horizontally or vertically. **Circle No. 34 on Reader Enquiry Form**

Digital readout refractometer. Anacon. The Model 42 provides instantaneously a digital LED display of refractive index and sample temperature. The readout can be calibrated in refractive index, percentage, degrees Brix or any other relevant unit and produces a repeatability of ± 0.0002 RI. Provision of external water bath connections allow sample temperature control. Size: 13×6×10 in; weight 15 lb. **Circle No. 35 on Reader Enquiry Form**

Automatic audiometer. B & K Laboratories. The Type 1800 Audiometer will provide extremely accurate and reliable automatic recordings in less than 10 min per individual, is for Bekesy type audiometry and provides a series of pure tone signals at seven different frequencies from 500 to 8000 Hz, either pulsed or continuous. **Circle No. 36 on Reader Enquiry Form**

In vitro Bleeding Time Recorder. Payton Associates. The BTR-400 replaces the traditional *in vivo* method eliminating some of the limitations and undesirable aspects e.g. trauma, repeated incision, and scarring. It also minimises the risk of infection. Simultaneous determinations of up to four different blood samples are possible. Blood is pumped at a constant rate through tubes, near the end of each of which is a minute orifice. Drops of blood fall through a photo-sensitive detector and this information is translated into bleeding time, stored in a memory module, and displayed via a 4-way selector switch on a digital (LED) counter. The Payton system also facilitates mixing experiments and provides ready access to the hemostatic plug for further study. **Circle No. 37 on Reader Enquiry Form**



Payton BTR-400

Miniature cryogenerator. Pye Unicam. The type MC80 Mini-Cooler is an extremely compact, lightweight cryogenerator able to produce temperatures down to 80 K within 15 min.

The MC80 measures 38 cm high and weighs 6 kg. Operating on the Philips-Stirling principle, the MC 80 produces a cold output, approximately liquid nitrogen temperature, at a copper 'cold finger' normally situated inside a transparent bell-jar vacuum head; there is provision for pneumatic lines and electrical connections to be made through the vacuum wall allowing observation of experiments carried out in the bell-jar. **Circle No. 38 on Reader Enquiry Form**

Resonant optical scanners. Techmation. Operating on the oscillating torsion bar (bearingless) principle, these motors exhibit an extremely long mechanical life and are designed for use in applications where a single frequency between 100 Hz and 2 kHz, fixed scanning angle and extreme reliability are required. They show a high order of amplitude stability (0.6% per °C) and are supplied complete with precision mirror. **Circle No. 39 on Reader Enquiry Form**

High versatility SEM/X-ray system. Philips. A high-performance scanning electron microscope PSEM 500 interfaced with a fully-focusing wavelength dispersive X-ray spectrometer, the PSEM 500X, not only permits analysis of X-rays from $Z=5$ to $Z=92$ but is the first such system to use the vertical spectrometer orientation—a configuration which offers the highest mechanical reproducibility—without the need for an optical microscope. Instead, a new standard in specimen height accuracy and reproducibility is achieved via the unique, fully-eucentric goniometer coupled with its 4.6 mm fine Z-adjustment (in 0.2 μ m steps) and the large (210 mm) Rowland circle of the spectrometer. An extremely high-vacuum system (10^{-6} torr, inclusive of the spectrometer assembly) vastly improves practical light-element detection. Thus, absorption of 'soft' X-rays from light elements, due to specimen contamination, is seldom encountered. The same vacuum system, without modification, allows operation with optional higher-brightness sources—a particularly valuable facility for excitation of X-rays from light elements. The standard 50 kV electron optics of the PSEM 500 is ideal, when using an energy dispersive X-ray detection system, for unambiguous excitation of K-line X-rays from heavier elements. The larger excited volume of X-rays, when using 50 kV electrons, can also facilitate easier trace-element detection. The energy dispersive and wavelength dispersive spectrometers can be in operation at the same time, thus giving an optimum choice of the characteristics of each type of spectrometer. **Circle No. 40 on Reader Enquiry Form**

CO₂ Incubator. Assab. In the T304GF, temperature is controlled within the range +25 °C to +75 °C to a tolerance of ± 0.3 °C throughout the cabinet. The CO₂ level, ranging from 0 to 25%, is continuously analysed and controlled through the use of a thermal conductivity sensor which responds rapidly to changes in CO₂ as small as 0.1%. This system ensures the most economical use of CO₂ and rapid recovery of the preset level following door opening. Humidity is produced by the controlled recirculation of the cabinet CO₂/air mixture through a water bath. A polymer foil hygrometer, accurate to $\pm 2.0\%$ RH, senses the relative humidity and operates the circulating pump accordingly. Both temperature and CO₂ control centres are solid state plug-in units which can be easily replaced. Controls are provided to set required levels which are displayed on digital meters. If required, visual or audible high/low alarms can be specified for temperature and CO₂ functions. Dimensions: 800 × 600 × 600 mm.

Circle No. 41 on Reader Enquiry Form

Scanning electron microscope. Philips. The SEM 501 has a guaranteed 70 Å resolution which can be consistently achieved and often exceeded and uses a three lens optics achieving a wide range of illuminating conditions. Its illumination efficiency, smallest spot size of 40 Å and low magnetic field at the specimen all combine to give high resolution images. Specimen positioning, at any magnification within the standard $\times 4$ to $\times 160,000$, is easily attained. The SEM 501 incorporates TV rate imaging, 30 kV electron optics, micron marker, independent number of lines and line time selection, high resolution photomonitor, digital display of magnification, Y modulation, a modular specimen rotation unit.

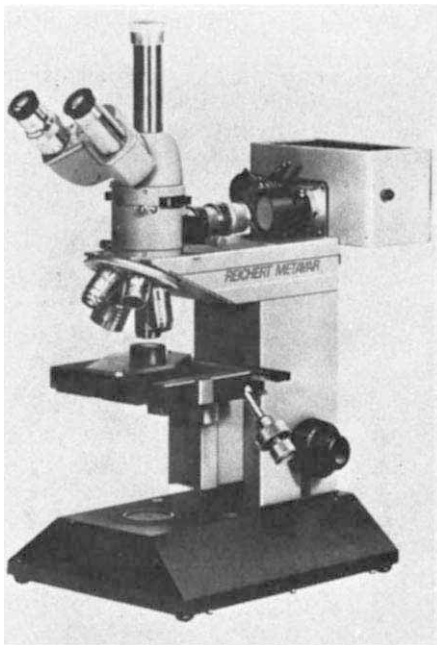
Circle No. 42 on Reader Enquiry Form

Electronic motionless switch. Siemens. A reliable, long-lived piezo-electric motionless switch for the direct control of ICs. The fully encapsulated switches can be used separately or grouped to construct switch panels and can be operated off symmetrical or asymmetrical voltages between 4 and 30 V, generates a current of 60 mA (adequate for miniature relays) for roughly 0.3 s under a brief pressure of around 150 g. In contrast to inductive proximity switches, this switch must be actuated by a defined pressure. A red, green or yellow light-emitting diode under a cap, which can be removed for the insertion of inscriptions, indicates the switch status.

Circle No. 43 on Reader Enquiry Form

Metallurgical microscope. Reichert-Jung. The MetavaR microscope has a series of variants which enable all known metallurgical examination techniques to be undertaken and uses an identical stand throughout the range but alternative optics and illuminators depending on the technique. The incident light equipment is available in three options either for bright field polarisation, dark field or interference contrast. They are easily and accurately fitted or interchanged between stand or viewing tube. The S is for polarised light and bright field examination, the U is for examinations in bright field, dark field and polarised light; and the IK is for interference contrast, polarised light and bright ground examinations. Supplementary equipment for the series includes dual viewing projection screen, camera systems and drawing apparatus.

Circle No. 44 on Reader Enquiry Form



Reichert-Jung MetavaR metallurgical microscope

Uric Acid Clinical Diagnostic Kit. Pierce Chemical. The kit uses a unique method for quantitative serum uric acid determination. Its direct serum procedure eliminates deproteinisation, and its unique two-tube method eliminates interferences from common drugs and native serum constituents. Absorbance values are linear to 20 mg dl⁻¹. The test can be run in a little over 35 min and colour development takes place within 15 min at room temperature. The final colour is stable for 90 min. The uricase is stable refrigerated; all other constituents are stable at room temperature.

Circle No. 45 on Reader Enquiry Form

Automatic sampling system. Hewlett-Packard. Batches of up to 60 samples can now be analysed automatically in sequence, and in replication if desired, by HP1084A and HP1082A processor-controlled liquid chromatographs. Only the sample amount injected is consumed by the system, making it possible to use micro-vials where only small samples are available.

A detachable unit containing 60 glass vials is located on top of the liquid chromatograph. A variable-volume sample injector, a standard part of the chromatograph, then samples each vial in numerical sequence. The injection volume can be pre-set manually at any point between 10 and 200 μ l.

Circle No. 46 on Reader Enquiry Form

Helium liquefier refrigerator. Sulzer. The Turbocryofridge TCF100, has a choice of two oil-free compressors, enabling a large number of plant variants to be achieved. By using the larger compressor and the cold box to its full extent with liquid nitrogen pre-cooling, it is possible to increase the minimum capacity of 10 l He/h (40 W at 4.5 K) to 80 l He/h (180 W at 4.5 K). For the generation of the necessary refrigerating capacity, the cold box is equipped with one or two turbo-expanders, each with dynamic gas bearings which operate at room temperatures. Braking is by a direct-coupled, single-stage centrifugal compressor.

Circle No. 47 on Reader Enquiry Form

TV attachment for light microscopes. Teletronics. A new closed-circuit TV attachment for multi-purpose microscopes, enables many people to view the same microscope slide simultaneously. The new system comprises a mini-CCTV camera, a 9-inch black-and-white TV monitor, and an attachment to link the camera to the microscope. Suitable for use with routine/research/biological microscopes having a sleeve of 23.2 mm inside diameter, the system gives users several options: camera-to-monitor cable distance can be from 1.5 to 100 m; the high-resolution 9-inch monitor can receive input from up to three cameras; and the monitor can be augmented by up to 10 additional monitor screens.

Circle No. 48 on Reader Enquiry Form

Automatic Colony Counter. Oriel Scientific. The Lab-Counter is an automated system giving fast and accurate counts of various types of samples both translucent and opaque. More than 700 samples per hour can be counted. The following samples can be taken by using the appropriate sample holder: microscopic slides, petri dishes 50–100 mm, tissue culture flasks, photographic films, filter dishes or photographs.

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